(51) International Patent Classification 6 :		(11) International Publication Number:	WO 98/04913
G01N 33/00, G06F 17/50	A1	(43) International Publication Date:	5 February 1998 (05.02.98)

(21) International Application Number: PCT/US97/13008

(22) International Filing Date: 24 July 1997 (24.07.97)

(30) Priority Data:
60/022,890   25 July 1996 (25.07.96)   US
60/032,786   6 December 1996 (06.12.96)   US
Not furnished   30 June 1997 (30.06.97)   US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: MOLECULAR MODEL FOR VLA-4 INHIBITORS

(57) Abstract

Pharmacophore models of VLA-4 inhibitors, methods of identifying novel inhibitors and novel inhibitors identified by these methods.

Intern   tal Application No
PCT/US 97/13008

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WO 9110140 A	11-07-91	AU 7034791 A	24-07-91
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		US 5459077 A	17-10-95

- 2 -

and/or their action still remain elusive and no desirable
antagonists have yet been discovered to inhibit or modulate
their activity.

5   Additionally, often certain agonists or antagonists of a
particular cell receptor are known, however, there remains a
need for methods of identifying new inhibitors, new molecular
entities and methods to quickly and effectively determine
whether a particular compound possesses a desired
pharmacological activity.

10   Cell adhesion is one of the fundamental mechanisms
underlying numerous biological phenomena, such as, for example,
the adhesion of hematopoietic cells to endothelial cells, and
the subsequent migration of those hematopoietic cells out of
the blood vessels and to the site of injury. Thus, cell
15 adhesion is known to play a role in numerous pathologies such
as inflammation and immune reactions.

α4β1 integrin, also known as very late antigen -4 ("VLA-
4"), is a leukocyte cell surface receptor that participates in
a wide variety of both cell-cell and cell-matrix adhesive
20 interactions. It serves as a receptor for the cytokine-
inducible endothelial cell surface protein, vascular cell
adhesion molecule-1 ("VCAM-1"), as well as to the extracellular
matrix protein fibronectin. Results of several in vivo
experiments suggest that the inhibition of VLA-4 dependent cell
25 adhesion may prevent, inhibit or alter several inflammatory and
autoimmune pathologies.

In order to identify the minimum active amino acid
sequence necessary to bind VLA-4, Komoriya et al. synthesized a
variety of overlapping peptides based on the amino acid
30 sequence of the CS-1 region (the VLA-4 binding domain) of a
particular species of fibronectin. ("The Minimal Essential

SUBSTITUTE SHEET (RULE 26)

- 3 -

Sequence for a Major Cell Type-Specific Adhesion Site (CS1)
Within the Alternatively Spliced Type III Connecting Segment
Domain of Fibronectin Is Leucine-Aspartic Acid-Valine", J.
Biol. Chem., 266 (23), pp. 15075-79 (1991)). They identified an
5   8-amino acid peptide, Glu-Ile-Leu-Asp-Val-Pro-Ser-Thr, as well
as two smaller overlapping pentapeptides, Glu-Ile-Leu-Asp-Val
and Leu-Asp-Val-Pro-Ser, that possessed inhibitory activity
against FN-dependent cell adhesion. These results suggested
that the tripeptide Leu-Asp-Val was the minimum sequence for
10   cell-adhesion activity. It was later shown that Leu-Asp-Val
binds only to lymphocytes that express an activated form of
VLA-4, thus casting doubt on the utility of such a peptide in
vivo. (E.A. Wayner et al., "Activation-Dependent Recognition by
Hematopoietic Cells of the LDV Sequence in the V Region of
15   Fibronectin", J. Cell. Biol., 116(2), pp. 489-497 (1992)).
However, certain larger peptides containing the LDV sequence
were subsequently shown to be active in vivo (T. A. Ferguson et
al., "Two Integrin Binding Peptides Abrogate T-cell-Mediated
Immune Responses In Vivo", Proc. Natl. Acad. Sci. USA, 88, pp.
20   8072-76 (1991); and S. M. Wahl et al., "Synthetic Fibronectin
Peptides Suppress Arthritis in Rats by Interrupting Leukocyte
Adhesion and Recruitment", J. Clin. Invest., 94, pp. 655-62
(1994)).

       A cyclic pentapeptide, Arg-Cys-Asp-TPro-Cys
25   (wherein TPro denotes 4-thioproline), which can inhibit both
VLA-4 and VLA-5 adhesion to FN has also been described. (See,
e.g., D.M. Nowlin et al. "A Novel Cyclic Pentapeptide Inhibits
$\alpha4\beta1$ and $\alpha5\beta1$ Integrin-mediated Cell Adhesion", J. Biol. Chem.,
268(27), pp. 20352-59 (1993); and PCT publication
30   PCT/US91/04862). This pentapeptide was based on the tripeptide
sequence Arg-Gly-Asp from FN which had been known as a common

SUBSTITUTE SHEET (RULE 26)

- 4 -

motif in the recognition site for several extracellular-matrix proteins.

Examples of other VLA-4 inhibitors have been reported, for example, in copending United States patent
5   application S.N. 08/376,372, specifically incorporated by reference herein. USSN 376,372 describes linear peptidyl compounds containing β-amino acids which have cell adhesion inhibitory activity. International patent applications WO 94/15958 and WO 92/00995, specifically incorporated by
10 reference, describe cyclic peptide and peptidomimetic compounds with cell adhesion modulating activity. International patent applications WO 93/08823 and WO 92/08464 (specifically incorporated by reference herein) describe guanidinyl-, urea- and thiourea-containing cell adhesion modulating compounds.
15 United States Patent No. 5,260,277 describes guanidinyl cell adhesion modulation compounds, and is also specifically incorporated herein.

As discussed above, it is desirable for several reasons to approach the discovery of new drugs in a rational as opposed to
20 a random manner. Thus, rather than making random modifications to a compound, one can rationally optimize the compound.

Ideally, a three dimensional model of the binding mode of inhibitors to a receptor is sought such that a correlation
25 between the structure of the compound and its effect on biological activity can be derived. Several general approaches exist for determining the three dimensional quantitative structure activity relationships of compounds and their receptors or ligands, including, but not limited to :
30 CATALYST™ (Greene et al., 1994, "Chemical Function queries for

- 5 -

Three dimensional database search", J. Chem. Inf. Comp. Sci., 34, 1297-1308) , DISCO (Martin Y.C., et al., 1993, "A Fast new approach to pharmacophore mapping and its application to dopinergic and benzodiazepine agonists", J. Comp. Aided Mol.

5   Design, 7, 83-102), COMFA(Cramer R.D., 1988, "Comparative molecular field analysis [CoMFA] 1. Effect of Shape on Binding of Steroids to Carrier Proteins", J. Am. Chem. Soc., 110, 5959-5967) , Apex3D (Golender, V.E. And Vorpagel, E.R., 1993, "Computer-assisted pharmacophore identification", Three

10   dimensional-QSAR in Drug Design:Theory, Methods and Applications, ESCOM Science Publ., Netherlands). Once a three dimensional model is built it can be useful in identifying novel compounds. For example, Kiyama et al. were able to identify novel AII antagonists based upon a three dimensional

15   model of known AII inhibitors. (1995, "Novel AII receptor antagonists. Design, synthesis, and in-vitro evaluation of dibenzo[a,d]cycloheptene and dibenzo[b,f]oxepin derivatives. Searching for bioisoteres of biphenyltetrazole using a Three dimensional search technique", J. Med. Chem., 38, 2728-2741).

20   In general, there are several fundamental forces which govern the molecular recognition between a drug and its receptor, including, for example, hydrogen-bonding forces, electrostatic and hydrophobic interactions. Until recently most descriptions of inhibitors have been based upon two

25   dimensional atomic topology diagrams which describe chemical structures (e.g. indole ring, carbonyl oxygen). Although these diagrams may be useful, they are somewhat limited in the information that they provide regarding the details of the biological activity of compounds. The availability of

30   additional information would aid chemists in identifying novel

- 6 -

compounds with a particular biological activity relatively quickly, cheaply and with a relatively high level of success.

An alternative to the two dimensional atomic topology approach (Greene et al., 1994, "Chemical Function queries for Three dimensional database search", J. Chem. Inf. Comp. Sci., 34, 1297-1308) describes compounds on the basis of chemical features which take into account the type of binding interaction of the chemical substructure.   (Figure 1 ; e.g. H-bonding donor, hydrophobe). One advantage of this approach is that it allows for a more general description of compounds, and accounts for its possible interactions with a receptor. The recognition that alternative chemical structures can present the same chemical features is central to drug discovery.

Examples of the use of the feature-based description of compounds to describe potent antagonists which differ in chemical structure but are similar in the chemical features they present exist. These include, for example, angiotensin converting enzyme antagonists(Sprague, 1994, "Building a hypothesis for Angiotensin Converting Enzyme Inhibition", MSI Inc., 16 New England Executive Park, Burlington, MA 01803) and A_2 antagonists (Sprague, 1994, "Building a hypothesis for AII Antagonism", MSI Inc., 16 New England Executive Park, Burlington, MA 01803).

Despite these advances, there remains a need for a model of a VLA-4 inhibitor which can be used to identify new specific inhibitors of cell adhesion, particularly for methods of identifying novel, specific inhibitors of VLA-4 cell adhesion.

The availability of additional information would aid those skilled in the art to identify novel compounds with a particular biological activity quickly, inexpensively, and with

SUBSTITUTE SHEET (RULE 26)

- 7 -

a relatively high level of success. Ideally, such methods
would allow practitioners to predict the inhibitory activity of
novel compounds which would provide useful agents for
treatment, alteration, prevention or suppression of various
5 pathologies mediated by cell adhesion and VLA-4 binding.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a model
of a VLA-4 inhibitor, methods of identifying new inhibitors,
and new compounds which inhibit VLA-4 activity which map to the
10 model, which substantially obviate one or more of the problems
due to the limitations and disadvantages of the related art.

To achieve the features and advantages of the invention,
as embodied and broadly described herein, the present invention
relates to a three dimensional pharmacophore model of a
15 compound having VLA-4 inhibitory activity. The claimed model
1 comprises certain features defined by the following tolerance
and three dimensional coordinates x, y and z. Specifically the
model comprises NEG ("N")

	Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
N	NEG	-8.564	1.564	-0.236	1.702

- 8 -

and at least three features selected from the group consisting of

	Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
1	HBA-1	-1.276	-1.259	-1.47	1.702
	HBA-2	-2.323	1.539	-1.35	1.702
2	HBD-1	6.693	1.988	-0.168	1.702
	HBD-2	7.217	0.939	2.630	2.302
3	HYD2	2.777	-1.061	-1.1501	1.702
4	HYD3	-3.803	-4.061	0.270	1.702
5	HYD4	9.377	2.219	1.050	1.702
6	HYD5	8.677	4.439	-1.330	1.702
7	HYD6	-9.123	-1.501	1.110	1.702

The coordinates of the claimed models define the relative relationship between the features, and therefore those skilled

5   in the art will readily recognize that the specific coordinates are dependent upon the particular coordinate system used, and thus, although rotation or translation of these coordinates may change the specific values of the coordinates, the coordinates will, in fact, define the claimed models.

10   Those skilled in the art should recognize that the claimed models are not without standard error. Thus, the claimed models are intended to encompass any model comprising the identified features and having a root mean square of equivalent features of less than about 2Å.

SUBSTITUTE SHEET (RULE 26)

- 9 -

More specifically, the model 1 of the invention comprises the negative ionizable feature " NEG", and at least four features selected from the group consisting of features 1-7. In other embodiments, the model may encompass 5-7 of the features, in addition to NEG.

In other embodiments, the applicant's invention relates to compounds which "map" to the claimed model. As used herein, the terms "map" and "fit" are used interchangeably to denote the correspondence between some or all of the features in a hypothesis and the chemical substructure of a particular conformer of a compound that satisfy those features, as computed by "catalyst" ("Hypothesis in Catalyst," MSI Inc., New England Executive Park, Burlington, MA 01803; Greene, I., 1994, J. Chem. Inf. Sci., "Chemical Function Queries for 3D Database Search," 34, 1297-1308). In additional embodiments, compounds having an IC_{50} value in a VLA-4 direct binding assay in the range of from about 100 μm to about 1 μm, and which comprise features which map to NEG, and an additional 3-7 features of the model, are encompassed.

In yet other embodiments, applicants have discovered novel methods for identifying chemical compounds having an IC_{50} value in a VLA-4 direct binding assay in the range of from about 100 μM to about 1 μM. The methods of the invention generally encompass selecting an experimental compound structure to be evaluated for VLA-4 inhibitory activity. The three dimensional structure of said compound is then obtained, and the structure of the experimental compound is then superimposed upon the VLA-4 model of the invention and evaluated to determine if the experimental compound "fits" the model. If the experimental compound fits the model, it is

- 10 -

then tested in a direct binding assay to determine whether or
not said experimental compound has the desired inhibitory
activity. The compounds of the invention preferably have an
inhibitory activity in the range of about 100 μM to about 0.5
5 nM, preferably of less than about 50 μm, more preferably less
than about 500 nM, and most preferably, less than about 50 nM.

In yet other embodiments, the claimed invention relates to
model 2, a three dimensional pharmacophore model of a compound
having VLA-4 inhibitory activity. Model 2 comprises Neg ("N")
10 as defined below, and at least four of features 1 through 8.

	Feature	x Å	y Å	z Å	tolerance Å
N	NEG	5.19	2.48	-0.84	1.5
1	HBA1-1	2.625	0.078	-0.451	1.5
	HBA1-2	1.434	2.840	-0.448	1.5
2	HBA2-1	6.038	-1.968	-0.039	1.5
	HBA2-2	8.314	-2.560	1.832	1.5
3	HBD-1	-6.17	-0.82	0.767	1.5
	HBD-2	-6.606	-3.3	2.412	1.5
4	HYD2	-1.126	-0.54	1.532	1.5
5	HYD3	1.054	-3.780	-2.528	1.5
6	HYD4	-8.786	-1.3	1.972	1.5
7	HYD5	-8.786	-0.580	-0.788	1.5
8	HYD6	8.594	2.12	-3.428	1.5

Preferably, the model comprises at least 5 to 8 of the
features 1-8 of Model 2. Additionally, applicants invention
relates to compounds which fit Model 2 and have features which
map to between 4 and 8 of the features of Model 2. The
15 compounds of the invention preferably have an inhibitory

SUBSTITUTE SHEET (RULE 26)

- 11 -

activity in the range of about 100 μM to about 50 nM,
preferably of less than about 50 μm, more preferably less than
about 500 nM, and most preferably, less than about 50 nM.

In other embodiments, the claimed invention relates to
5   methods for identifying chemical compounds having VLA-4
inhibitory activity using Model 2 in a manner similar to that
described above for Model 1, as well as to compounds obtained
by the claimed methods.

In still other embodiments, the claimed invention
10   relates to a third three dimensional pharmacophore model of a
compound having VLA-4 inhibitory activity. Model 3 comprises
the following features:

Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
Carboxyl C	-3.131	-2.023	2.824	1.2
Carboxyl O1	-3.513	-0.027	4.108	0.9
Carboxyl O2	-1.487	-0.895	4.167	0.9
Carbonyl C	-2.241	2.730	0.315	0.9
Carbonyl O	-3.067	3.241	1.064	0.9

As discussed above in relation to Models 1 and 2, the
invention also encompasses methods for identifying desired
15   compounds using Model 3, as well as novel compounds which map
to Model 3. Preferably, the novel compounds encompassed by the
claims have the preferred IC_{50} values discussed above.

It is to be understood that both the foregoing general
description and the following detailed description are
20   exemplary and explanatory and are intended to provide further
explanation of the invention as claimed. The accompanying

- 12 -

drawings are included to provide a further understanding of the invention and are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention, and together with the description serve to explain the

5   principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Atom and Feature-based description of the compound M14. The atom-based description shows the three dimensional arrangement of atoms and bonds of M14.

10   Figure 2. The overlay, as computed by Catalyst, between M14 and the claimed VLA-4 Model 1. The features of the model have been labeled.

Figure 3 (a) - (x). The chemical structures of compounds which map to the VLA-4 model 1.

15   Figure 4. The overlay as computed by Catalyst between M2 and the claimed VLA-4 Model 2.

Figure 5. The correspondence of Model 3 and Gln-Ile-Asp-Ser-Pro region (residues 38-42)of the VCAM structure (Bernstein, F., 1977, "The Protein Databank: a computer-based archival for

20   macromolecular structures," J. Mol. Biol., 112, 535-542 ; Brookhaven Code 1VCA).

Figure 6. Overlay of diphenylurea containing compounds from the Cambridge Crystallographic Database (Code names PPESIR, KUHWHIT, SILVOY, GIMROJ10, GIMRUP10, GIMSAW10, SALTOW,

25   SILTUC01).   (Cambridge Crystallographic Data Center, 12 Union

- 13 -

Road, Cambridge, CB21EZ, U.K.) A top view and end-on-view of
the overlaid compounds are shown.

Figure 7. A schematic representation of our search procedure
for identifying diphenylurea mimetics. This involves defining
5   and extracting the compound DPUREA from the Cambridge
crystallographic database, and defining its shape using the
program catShape. This is defined in terms of a mask which
represents the 3 principal axes of the molecule and its volume.
This mask is then used to search for other molecules in a
10 Catalyst database with similar shapes and volumes.

Figure 8. Examples of diphenylurea mimetics extracted from a
multiconformational database of amine containing caps extracted
from the ACD.

Figure 9. The overlay of the Gln-Ile-Asp-Ser-Pro portion of
15 the VCAM X-ray structure (Residue Number 38-41) and a
truncated version of the VLA-4 model 1

Figure 10. The overlay, as computed by Catalyst, between M2
and the VLA-4 model 1.

Figure 11. The conversion of the compound M5 into M6, a VLA-4
20 inhibitor. The overlay of M6 with the VLA-4 model 1 is shown.

Figure 12. Comparison of the X-ray structures of Leucine
Aminopeptidase (Brookhaven Code 1BPM) and VCAM (Brookhaven Code
1VCA.)

Figure 13. Two potent VLA-4 inhibitors (M13 and M17) with the
25 Model 3.

- 14 -

Figure 14. The fit of two novel compounds to the Model 3. These were identified by searching through a commercially available database of scaffolds using Catalyst.

Figure 15.(a), 15(b), 15 (c). A list of preferred compounds of
5   the claimed invention.

DETAILED DESCRIPTION

Reference will now be made in detail to the present preferred embodiments of the invention, examples of which are illustrated in the accompanying drawings.

10   Applicants have invented 3-dimensional models which consist of the chemical features needed for a compound to inhibit the binding of ligands to the VLA-4 receptor. The models are generated from structure-activity data and are descriptions of chemical substructures or features that are
15   important for biological activity within a class of molecules. Molecules that can present certain of the chemical features in a relative three dimensional orientation as described by the models are predicted to have VLA-4 inhibitory activity as measured by a VLA-4 direct binding assay (DBA) and may
20   therefore have therapeutic potential. The models are feature-based, describing compounds on the basis of chemical features which take into account the type of binding interaction of the chemical substructure (Greene et al., 1994, "Chemical Function queries for Three dimensional database search", J. Chem. Inf.
25   Comp. Sci., 34, 1297-1308).   (Figure 1 ; e.g. H-bonding donor, hydrophobe). One advantage of this approach is that it allows for a more general description of compounds, and accounts for possible interactions with a receptor. The recognition that alternative chemical structures can present

- 15 -

the same chemical features is central to drug discovery.
Examples exist of the use of the feature-based description of
compounds to describe potent antagonists which differ in
chemical structure but are similar in the chemical features
5   they present. These include, for example, angiotensin
converting enzyme antagonists and AII antagonists. (Sprague,
1994, "Building a hypothesis for AII Antagonism", MSI Inc., 16
New England Executive Park, Burlington, MA 01803).

The models of the invention provide those skilled in the
10   art with a tool for discovering novel VLA-4 inhibitors, and
thus, can be used to evaluate compounds prior to synthesis as
to their ability to inhibit ligand binding to the VLA-4
receptor, or to design new compounds. The compounds being
evaluated are referred to herein as "experimental compounds".
15   More specifically, those skilled in the art will find that the
claimed models can be used in conjunction with a computational
computer program, such as, for example, Catalyst™ , to search
through chemical databases for chemical substructures of
"experimental compounds" that might fit all or part of the
20   model, and use the information so gathered to determine whether
the experimental compound is likely to have VLA-4 inhibitory
activity. Additionally, the claimed invention can provide the
artisan with a tool to compare various experimental compounds
not only with the claimed model, but with other experimental
25   compounds. In other embodiments, those skilled in the art may
use the claimed invention in combination with other software
programs, such as, for example Denovo design software programs
(e.g. Leapfrog "Ligand-Based Design Manual", Tripos Inc., 1699
S. Hanley Road, St. Louis, Missouri 63144-2913) to identify

- 16 -

templates or chemical substructures which fit all or part of the model, and thereby determine the quality of the "fit". "Fit" is used herein to denote the correspondence between some or all of the features of an experimental compound to a

5   reference model.   In yet other embodiments, the claimed invention can be used by the artisan as a basis for intuitively designing novel VLA-4 inhibitors.

The claimed invention relates to a feature based three dimensional VLA-4 model which can be used to identify novel

10   VLA-4 inhibitors. Figure 2 shows the claimed VLA-4 model 1. As depicted, the model 1 consists of a set of features arranged in three dimensional space. Each feature is a definition of a chemical property of functional groups on molecules.   Thus, as illustrated in Figure 2, the relationship of chemical

15   structures and features is given. Complete definitions of these features have been published, and can easily be understood by one skilled in the art. See, for example, Greene, J., Kahn, S., Savoj, H., Sprague, P., and Teig, S., 1994, "Chemical Function Queries for Three dimensional Database

20   Search", J. Chem. Inf., and Comp. Sci., 34, 1297-1308, specifically incorporated herein by reference. The Cartesian coordinates of the claimed models can be defined mathematically by the x, y and z axes, and associated tolerance values. Unless otherwise stated, all Cartesian coordinates given herein are

25   measured in angstroms.

A.   PHARMACOPHORE MODELS

i) Model 1

The claimed model 1 comprises features defined by the following tolerance and three dimensional coordinates x, y and

30   z. Specifically, Model 1 comprises NEG ("N") as defined below

- 17 -

	Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
N	NEG	-8.564	1.564	-0.236	1.702

and at least three features selected from the group consisting of

	Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
1	HBA-1	-1.276	-1.259	-1.47	1.702
	HBA-2	-2.323	1.539	-1.35	1.702
2	HBD-1	6.693	1.988	-0.168	1.702
	HBD-2	7.217	0.939	2.630	2.302
3	HYD2	2.777	-1.061	-1.1501	1.702
4	HYD3	-3.803	-4.061	0.270	1.702
5	HYD4	9.377	2.219	1.050	1.702
6	HYD5	8.677	4.439	-1.330	1.702
7	HYD6	-9.123	-1.501	1.110	1.702

The claimed model, in certain broad embodiments comprises the feature referred to as "Neg", i.e. a negative ionizable
5   feature, and, in different embodiments, from three to seven of the seven features described above. It should be noted that, as used herein, the hydrogen-bond acceptor feature, HBA, Feature # 1, although encompassing both HBA-1 and HBA-2, is considered a single feature. Similarly, the HBD or hydrogen-
10   bond donor feature, Feature # 2, is discussed as a single

- 18 -

feature herein, although it encompasses both HBD-1 and HBD-2.
"Hyd" as used herein refers to the Hydrophobic features.

The coordinates of Model 1 and the other Models claimed
herein define the relative relationship between the features.

5   Furthermore, the coordinates are dependent upon the particular
coordinate system used, and those skilled in the art will
recognize that, although rotation or translation of these
coordinates may change the specific value of the coordinates,
they will in fact define the claimed Models. The claimed

10   Models are intended to encompass any model, after optimal
superimposition of the models, comprising the identified
features and having a root mean square of equivalent features
of less than about 2 Å. More preferably, the claimed model
encompasses any model comprising the identified features and

15   having a root mean square of equivalent features of less than
about 1.5 Å, and most preferably, less than about 1.0 Å.

The VLA-4 model can be used to evaluate the ability of a
compound to inhibit the binding of VLA-4 to its receptor. The
compound evaluated for inhibitory activity, the "experimental

20   compound" can be a novel structure designed using the claimed
model, or, alternatively can be a structure known in the art.
Using the claimed model and methods, and the teachings herein,
those skilled in the art can predict that an experimental
compound which "fits" or "maps" to the model will have VLA-4

25   inhibitory activity.

In practice, the claimed model can be used in a variety of
ways. For example, the claimed model can be used according to
the claimed methods to identify novel VLA-4 inhibitors. First,
one identifies an experimental compound to be evaluated for

30   VLA-4 activity. This can be done for example, by searching a
chemical database, or by modifying an existing compound.

SUBSTITUTE SHEET (RULE 26)

- 19 -

Alternatively, one can create a novel experimental compound. Those skilled in the art routinely utilize computer databases for searching, and computationally creating and/or modifying compounds.

5   After identifying an experimental compound to be evaluated for VLA-4 inhibitory activity, the three dimensional structure of the experimental compound is determined.   One can use computer programs such as, for example, Catalyst™ software, however, one is not limited to this software. By way

10   of example, the compound is drawn using the drawing tools in Catalyst™ and 3-dimensional conformations can then be generated using, for example, the Best conformer generation process with. energy set to 10 Kcal/molss and the maximum number of conformations generated being set to 250.   The model is then

15   fit to the experimental compound using tools which can compare the two structures, such as, for example, Compare within the ViewHypothesis workbench.

The "fit" can be calculated automatically, for example, by determining if the compound can map to the chemical features in

20   the model.   This is dependent on whether the compound has the necessary or desired functional groups, and also whether they can adopt the necessary three dimensional arrangement to fit the model.   The program can automatically report which features in the model are mapped by a compound. A "fit" as

25   used herein means that the experimental compound must include the negative ionizable feature, and at least three others of the 7 features in the model are mapped.

If the experimental compound fits or maps the model, then one can experimentally determine whether that compound has the

30   desired VLA-4 inhibitory activity by performing a direct binding assay (DBA). Those skilled in the art routinely

perform such assays, and can readily determine the activity of the experimental compound.

In preferred embodiments, the claimed invention encompasses VLA-4 inhibitors having a commercially useful

5   selectivity and specificity. These values may vary widely, however, are easily determined by those skilled in the art based upon the desired application of the inhibitor. In general, the inhibitors of the invention have an IC_{50} value of less than about 100 μm in a VLA-4 direct binding assay. More

10   preferably that value is less than about 50 μm, more preferably less than about 1μm, In yet more preferred embodiments, the VLA-4 inhibitors have an IC_{50} of less than about 500 nM, less than about 100 nM, and most preferably, less than about 50 nM.

Applicants claimed methods and compounds enable those skilled

15   in the art to predict and obtain VLA-4 inhibitors which have more desirable activities than those available in the art.

Applicants generated the claimed VLA-4 model as follows. A training set consisting of M16-01, M14, M18, M19 (Figures 3p, q, r and s respectively) was selected as representative of the

20   different families of active, known, VLA-4 inhibitors. The training set was converted to multiconformer models with Catalyst™ 3.1 (Catalyst Tutorial Manual, MSI Inc., 16 New England Executive Park, Burlington, MA 01803) using the Best conformer generation process with energy set to 10 Kcal/molss

25   and a maximum confs set to 250. These were used as input to the model generation program HIPHOP™ (HIPHOP Manual, MSI Inc., New England Executive Park, Burlington, MA 01803) as implemented in Catalyst™.

Applicants set the common features mode using the

30   default arguments for all parameters except the following. All

- 21 -

compounds were cited as principal by setting the principal
column to 2 for each. The MaxOmit feature was set to 0. The
spacing parameter was to 250 picometers, the max and min
parameters were set to 9, and the number of returned models was
5 set to 10. Applicants determined that Model 1 was
representative of a VLA-4 inhibitor.

In order to further validate the claimed methods and
model, applicants determined whether known VLA-4 inhibitors fit
the claimed model. Protein X-ray crystallography, a powerful
10 and commonly used experimental tool which provides structural
insight into the biological conformation of macromolecules, was
used to determine the crystallographic structure of vascular
cell adhesion molecule 1 (VCAM ; Brookhaven Code 1vca). This
is a known physiological ligand of the VLA-4 receptor, which
15 contains the sequence Ile-Asp-Ser. The Ile-Asp-Ser sequence is
homologous to the Leu-Asp-Val sequence from CS1 upon which the
peptidomimetics used in the claimed model were based. Peptides
based upon Leu-Asp-Val region have been shown to inhibit the
VLA-4-VCAM interaction. (Wang et al., 1995). Thus, applicants
20 hypothesized that VLA-4 antagonists, when they bind to VLA-4,
may mimic the structure of the Ile-Asp-Ser region of the VCAM
structure.

The overlay between the model and Ile-Asp-Ser portion of
VCAM is shown in Figure 2. As can be seen in Figure 2, the
25 Ile "maps" the hydrophobic feature HYD3 and the carboxyl group
of the Asp "maps" the negative ionizable feature NEG.
Additionally, the carbonyl of the Ile residue maps to the
hydrogen-bond acceptor feature. Thus applicants were able to
confirm their claimed model by the mapping of the binding
30 epitope of a known ligand of VLA-4 to the model.

   ii)   Model 2

- 22 -

The claimed Model 2, depicted in Figure 4, comprises features defined by the following tolerances and three dimensional coordinates. Specifically, Model 2 comprises the feature NEG ("N") and at least four other features selected from the remaining group of eight features.

Description of Model 2

	Feature	x Å	y Å	z Å	tolerance Å
N	NEG	5.19	2.48	-0.84	1.5

1	HBA1-1	2.625	0.078	-0.451	1.5
	HBA1-2	1.434	2.840	-0.448	1.5
2	HBA2-1	6.038	-1.968	-0.039	1.5
	HBA2-2	8.314	-2.560	1.832	1.5
3	HBD-1	-6.17	-0.82	0.767	1.5
	HBD-2	-6.606	-3.3	2.412	1.5
4	HYD2	-1.126	-0.54	1.532	1.5
5	HYD3	1.054	-3.780	-2.528	1.5
6	HYD4	-8.786	-1.3	1.972	1.5
7	HYD5	-8.786	-0.580	-0.788	1.5
8	HYD6	8.594	2.12	-3.428	1.5

Model 2 was generated as follows. A training set consisting of M21, M26, M23, M22, and M24, was selected, and used to build Model 2.

The training set was converted to multiconformer models with Catalyst™2.3.1 (Catalyst Tutorial Manual, Release 2.3, MSI Inc., 16 New England Executive park, Burlington, MA 01803) using the Best conformer generation process with energy set to 15 Kcal/molss and a maximum confs set to 255. These were used as input to the hypothesis generation program HIPHOP™ (Catalyst

- 23 -

Tutorial Manual, Release 2.3, MSI Inc., 16 New England
Executive Park, Burlington, MA 01803) as implemented in
Catalyst™ 2.3.1.

Hypothesis generation was carried out with the common

5   features mode set to using the default arguments for all
parameters except the following. All compounds were cited as
principal by setting the principal column to 2 for each. M21
was not permitted to miss any features, M20 was allowed to miss
up to two features, and all other compounds were permitted to

10   miss up to one feature of any generated hypothesis by setting
the MaxOmitFeatures columns to 0,2 and 1 respectively. The
spacing parameter was to 250 picometers, the max and min
parameters were set to 9, and the number of returned hypothesis
was set to 20. Applicants thus created 12 nine-featured models

15   of which the fourth highest ranking was determined to best
correlate the observed structure-activity data.

iii) Model 3

Applicants claimed Model 3 is based upon the discovery
that certain VLA-4 inhibitors may be involved in coordinating

20   to a metal in the VLA-4 ligands. Thus, applicants hypothesized
that scaffolds which can coordinate to the metal may be useful
to replace the scaffold of known VLA-4 inhibitors. The term
"scaffold" is used herein to describe a portion of the
chemical structure of VLA-4 inhibitors relating to the Leu-Asp-

25   Val portion of known VLA-4 inhibitors. Applicants have
discovered that the scaffold on known inhibitors can be
replaced with new or different chemical substructures, thereby
creating novel VLA-4 inhibitors.

Applicants based the claimed Model 3 upon VCAM, a known

30   VLA-4 ligand, which was used to search for scaffolds which can

- 24 -

coordinate to metals. The elements of Model 3, defined below, correspond to specific atom types, whereas Models 1 and 2 refer to chemical features.

Model 3 comprises the following five features:

Feature	x Å	y Å	z Å	tolerance Å
Carboxyl C	-3.131	-2.023	2.824	1.2
Carboxyl O1	-3.513	-0.027	4.108	0.9
Carboxyl O2	-1.487	-0.895	4.167	0.9
Carbonyl C	-2.241	2.730	0.315	0.9
Carbonyl O	-3.067	3.241	1.064	0.9

5   Applicants generated Model 3 by first defining key moieties of VCAM, a known VLA-4 ligand, which interact with metals. The backbone carbonyl group of residue Gln38 and the carboxyl group of Asp40 were identified. Applicants extracted the three-dimensional coordinates of the atoms included in the

10   two moieties from the VCAM structure (Brookhaven Code 1VCA), and the then translated the two moieties into a searchable model using the Catalyst program.

B.   METHODS USING CLAIMED MODELS

       The model of the invention provides those skilled in the

15   art with a tool for discovering novel VLA-4 inhibitors, and thus, can be used to evaluate compounds prior to synthesis as to their ability to inhibit ligand binding to the VLA-4 receptor, or to design new compounds. The compounds being evaluated for VLA-4 inhibitory activity are referred to herein

20   as "experimental compounds".

- 25 -

The VLA-4 models can be used to evaluate the ability of a compound to inhibit VLA-4. The compound evaluated for inhibitory activity, the "experimental compound" can be a novel structure designed using the claimed model, or, alternatively,

5   can be a structure known in the art. Using the claimed models and methods, and the teachings herein, those skilled in the art can predict that an experimental compound which "fits" or "maps" to the models will have VLA-4 inhibitory activity.

In practice, the claimed models can be used in a variety

10 of ways. For example, the claimed models can be used according to the claimed methods to identify novel VLA-4 inhibitors. First, one identifies an experimental compound to be evaluated for VLA-4 activity. This can be done for example, by searching a chemical database, or by modifying an existing compound.

15 Alternatively, one can create a novel experimental compound. Those skilled in the art routinely utilize computer databases for searching, and computationally creating and/or modifying compounds and building compounds deNovo (e.g. LeapFrog "Ligand-Based Design Manual",Tripos Inc., 1699 S. Hanley Road, St.

20 Louis, Missouri 63144-2913).

After identifying an experimental compound to be evaluated for VLA-4 inhibitory activity, the three dimensional structure of the experimental compound is determined. One can use computer programs such as, for example, Catalyst™

25 software, however, one is not limited to this software. By way of example, the compound can be drawn using the drawing tools in Catalyst™ and three-dimensional conformations can then be generated using, for example, the Best conformer generation process with energy set to 10 kcal/mols and the maximum number

30 of conformations generated being set to 250. The experimental compound is then fit to the models using tools which can

SUBSTITUTE SHEET (RULE 26)

- 26 -

compare the two structures, such as, for example, Compare within the ViewHypothesis workbench.

The "fit" can be calculated automatically, for example, by determining if the compound can map to the chemical features in
5   the models. This is dependent on whether the compound has the necessary or desired functional groups, and also whether they can adopt the necessary three dimensional arrangement to fit the model. The program can automatically report which features in the models are mapped by a compound.

10   If the experimental compound fits or maps any of the models, then one can experimentally determine whether that compound has the desired VLA-4 inhibitory activity by performing a direct binding assay (DBA). Those skilled in the art routinely perform such assays, and can readily determine
15   the activity of the experimental compound.

In preferred embodiments, the claimed invention encompasses methods of identifying VLA-4 inhibitors having a commercially useful selectivity and potency. These values may vary widely, however, are easily determined by those skilled in
20   the art based upon the desired application of the inhibitor. In general, the inhibitors of the invention have an IC_{50} value of less than about 100 µm in a VLA-4 DBA. More preferably the claimed methods can be used to identify compounds having an IC_{50} that value is less than about 50 µm, more preferably less
25   than about 1µm. In yet more preferred embodiments, the VLA-4 inhibitors have an IC_{50} of less than about 500 nM, less than about 100 nM, and most preferably, less than about 50 nM. Applicants' claimed methods and compounds enable those skilled in the art to predict and obtain VLA-4 inhibitors which have
30   more desirable activities than those available in the art.

- 27 -

A method for the Discovery of DiphenylUrea Replacements in our VLA4 Inhibitors

A series of VLA-4 inhibitors previously reported, i.e. USSN 08/376,372, comprise a diphenylurea "cap," and a
5   "scaffold" as discussed above. The "cap" is used herein to describe a part of the chemical structure of VLA-4 inhibitors; generally, the cap on known inhibitors can be replaced with new or different chemical substructures, and thereby create novel VLA-4 inhibitors. The term cap is used herein to describe
10   replacements of the N-terminal cap, i.e., 2'- tolylureidophenylacetyl (M1 ; Figure 3a), with other chemical structures.

Applicants have invented methods of identifying mimetics of the diphenylurea cap of known VLA-4 inhibitors, which can
15   be used to identify novel VLA-4 inhibitors. The claimed methods provide those skilled in the art with an effective and valuable tool to identify alternate cap structures , i.e. other than diphenyl analogs of diphenylurea, and hence can be useful for the identification of novel inhibitors.

20   The catShape program(Catalyst 3.1 Installation Guides and Release Notes, San Diego, Molecular Simulations Inc., 1996) is able to compare the shapes and volumes of compounds to each other. Applicants using the claimed methods, utilized this program to identify compounds that are similar to diphenylurea,
25   (i.e. the "cap" on certain known VLA-4 inhibitors), and which are suitable for combinatorial chemistry.

The X-ray crystal structure of diphenylurea(Code name DPUREA) was extracted from the Cambridge Crystallographic Database(October 1991 release; Cambridge Crystallographic Data
30   Center, 12 Union Road, Cambridge CB21EZ, U.K.). There are other

- 28 -

diphenylurea containing compounds with very similar structures
(Figure 6) in the database (Code : PPESIR, KUHWHIT, SILVOY,
GIMROJ10, GIMRUP10, GIMSAW10, SALTOW, SILTUC01). After
converting the format of the file from Cambridge Database

5   format to MDL SD format (MDL Information Systems, 14600
Catalina Street, San Leandro, CA94577), the DPUREA it then used
as input to the program catShape. This compound is used to
search for compounds of similar shapes and volumes (Figure 7)
in a database of molecules (e.g., Available Chemical Directory

10   ; MDL Information Systems, 14600 Catalina Street, San Leandro,
CA 94577). These chemical databases are converted into Catalyst
databases by reading them into Catalyst 3.1 and building
conformational models for each compound using the Fast
conformer generation process with energy set to 10 kcal/mols,

15   the maximum number of retries set to 100 and the maximum number
of conformations set to 250. The catShape method involves
calculating the size of the three principal orthogonal axis of
each molecule in the database, together with the volume of the
compound, and comparing each of these to that of the

20   diphenylurea. The parameters are set to the default settings as
described on page RN-10 of the Catalyst 3.1 Installation Guide
and Release Notes (Molecular Simulations Inc., 1996, 9685
Scranton Road, San Diego). We used the default tolerances of
20% to the principal axes during the search procedure.

25   Thus, in certain embodiments the claimed invention
encompasses methods for identifying mimetics of diphenylurea,
specifically, methods of identifying non-related mimetics. The
methods of the invention thus encompass methods of identify
VLA-4 inhibitors having as a substructure a diphenylurea

30   mimetic.

- 29 -

As discussed above, the methods of the invention involve as a first step the selection of an experimental compound to be evaluated for VLA-4 inhibitory activity, and determining whether said experimental compound contains a chemical

5   substructure of similar shape and volume to diphenylurea as defined in the art, (i.e. by catShape or other programs). In certain embodiments, the method involves instead a determination of whether a three dimensional substructure of said experimental compound maps the features 2, 3, 5 and 6 in

10   the VLA-4 Model 1. A three dimensional structure of the entire experimental compound is then obtained, either experimentally, or computationally, which is then mapped to the VLA-4 Model-1 Neg and feature 1. If the experimental compound has a substructure which contains an atom within about 0.5 to about

15   3Å, more preferably about 1 to about 2 Å, and most preferably, about 2 Å of any of features 2, 3, 5 or 6, then the experimental compound is predicted to have VLA-4 inhibitory activity. The experimental compounds containing the urea mimetics (Figure 8) can then be tested using a DBA, as

20   discussed above.

Using the claimed three dimensional models those skilled in the art can identify both novel chemical entities which fit the model and have VLA-4 inhibitory activity, as well as identify known compounds having the desired activity which were

25   not previously known to be VLA-4 inhibitors. The following examples further illustrate various embodiments of the claimed invention, and further enable those skilled in the art to practice the invention.

C.   Novel VLA-4 Inhibitors

- 30 -

(i) Inhibitors which map to Model 1

The claimed invention in certain broad embodiments encompasses novel compounds which have VLA-4 inhibitory activity. The claimed compounds fit the claimed models, have
5   surprisingly good inhibitory activity and thus can be used, for example, in pharmaceutical preparations for treatment of diseases and conditions involving the VLA-4 pathway.

Using the pharmacophore models and methods described in the foregoing sections, the applicants have discovered novel
10 VLA4 inhibitors which fit the models.

For example, preferred embodiments encompasses VLA4 inhibitory compounds represented by GB-1:
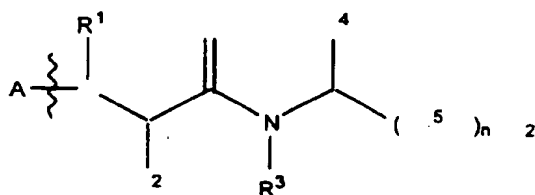
GB-1

15   A is selected from the group consisting of alkyl; aliphatic acyl optionally substituted with N-alkyl- or N-arylamido; aroyl; heterocycloyl; alkyl- or arylsulfonyl; aralkylcarbonyl optionally substituted with aryl; heterocycloalkylcarbonyl; alkoxycarbonyl; aralkyloxycarbonyl;
20 cycloalkylcarbonyl optionally fused with aryl; heterocycloalkoxycarbonyl; alkylaminocarbonyl; arylamino carbonyl and aralkylaminocarbonyl optionally substituted with bis(alkylsulfonyl)amino, alkoxycarbonylamino or alkenyl; alkylsulfonyl; aralkylsulfonyl; arylsulfonyl;
25 cycloalkylsulfonyl optionally fused with aryl;

SUBSTITUTE SHEET (RULE 26)

- 31 -

heterocyclylsulfonyl; heterocyclylalkylsulfonyl;
aralkoxycarbonyl; aryloxycarbonyl; cycloalkyloxycarbonyl;
heterocyclyloxycarbonyl; heterocyclylalkoxycarbonyl; mono- or
di-alkylaminocarbonyl optionally substituted with aryl;
5   (alkyl)(aralkyl)aminocarbonyl; mono- or di-
aralkylaminocarbonyl; mono- or di-arylaminocarbonyl;
(aryl)(alkyl)aminocarbonyl; mono- or di-
cycloalkylaminocarbonyl; heterocyclylaminocarbonyl;
heterocyclylalkylaminocarbonyl;
10   (alkyl)(heterocyclyl)aminocarbonyl;
(alkyl)(heterocyclylalkyl)aminocarbonyl;
(aralkyl)(heterocyclyl)aminocarbonyl;
(aralkyl)(heterocyclylalkyl)aminocarbonyl; alkenoyl optionally
substituted with aryl; alkenylsulfonyl optionally substituted
15   with aryl; alkynoyl optionally substituted with aryl;
alkynylsulfonyl optionally substituted with aryl;
cycloalkenylcarbonyl; cycloalkenylsulfonyl; cycloalkylalkanoyl;
cycloalkylalkylsulfonyl; arylaroyl, biarylsulfonyl;
alkoxysulfonyl; aralkoxysulfonyl; alkylaminosulfonyl;
20   aryloxysulfonyl; arylaminosulfonyl; N-arylurea-substituted
alkanoyl; N-arylurea-substituted alkylsulfonyl; cycloalkenyl-
substituted carbonyl; cycloalkenyl-substituted sulfonyl;
alkenoxycarbonyl optionally substituted with aryl;
alkenoxysulfonyl optionally substituted with aryl;
25   alkynoxycarbonyl optionally substituted with aryl;
alkynoxysulfonyl optionally substituted with aryl; alkenyl- or
alkynyl-aminocarbonyl optionally substituted with aryl;
alkenyl- or alkynyl-aminosulfonyl optionally substituted with
aryl; acylamino-substituted alkanoyl; acylamino-substituted
30   alkylsulfonyl; aminocarbonyl-substituted alkanoyl; carbamoyl-
substituted alkanoyl; carbamoyl-substituted alkylsulfonyl;

SUBSTITUTE SHEET (RULE 26)

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- 32 -

heterocyclylalkanoyl; heterocyclylaminosulfonyl; carboxyalkyl-
substituted aralkoyl; carboxyalkyl-substituted aralkylsulfonyl;
oxocarbocyclyl-fused aroyl; oxocarbocyclyl-fused arylsulfonyl;
heterocyclylalkanoyl; N',N'-alkyl, arylhydrazinocarbonyl;

5   aryloxy-substituted alkanoyl and heterocyclylalkylsulfonyl;
alkenyl, alkynyl, cycloalkyl, aryl-fused cycloalkyl,
cycloalkenyl, aryl, aryl-substituted alkyl ("aralkyl"), aryl-
substituted alkenyl or alkynyl, cycloalkyl-substituted alkyl,
cycloalkenyl-substituted cycloalkyl, biaryl, alkoxy, alkenoxy,

10 alkynoxy, aryl-substituted alkoxy ("aralkoxy"), aryl-
substituted alkenoxy or alkynoxy, alkylamino, alkenylamino or
alkynylamino, aryl-substituted alkylamino, aryl-substituted
alkenylamino or alkynylamino, aryloxy, arylamino, N-alkylurea-
substituted alkyl, N-arylurea-substituted alkyl,

15 alkylcarbonylamino-substituted alkyl, aminocarbonyl-substituted
alkyl, heterocyclyl, heterocyclyl-substituted alkyl,
heterocyclyl-substituted amino, carboxyalkyl substituted
aralkyl, oxocarbocyclyl-fused aryl and heterocyclylalkyl;

    $n=1-4$;

20 When R^3 is H, n=2-4; or when n=1, only R^3 or R^5 is H;
R^1 and R^4 are independently selected from the group consisting
of H, alkyl, aryl, aralkyl; alkyl optionally substituted with
cycloalkyl, cycloalkenyl, heterocycle, alkenyl, alkynyl,
alkoxyl, hydroxyl, halogen, aralkoxy, thioalkoxy, carboxy,

25 alkoxycarbonyl, carboxamide, amine, alkylsulfone, and
alkylsulfoxide;
R^2 is selected from the group consisting of H, alkyl: alkyl
optionally substituted with amine, cycloalkyl, alkylsulfone,
and alkylsulfoxide;

SUBSTITUTE SHEET (RULE 26)

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- 33 -

R^3 is selected from the group consisting of H, alkyl, and alkyl optionally substituted with aralkoxy, hydroxy;

X is selected from the group consisting of $-CH_2-$, S, O, NR^4, $NCOR^7$, and NSO_2R^7;

5   m is 3 or 4;

p is 3 or 4;

q and r are independently 1 or 2;

R^1 and R^2 may be taken together to form $-(CR^1R^2)_p-$, or $-(CR^1R^2)_qX(CR^1R^2)_r-$;

10   R^3 and R^4 may be taken together to form $-(CR^1R^2)_m-$ or $-(CR^1R^2)_qX(CR^1R^2)_r-$;

R^3 and R^5 may be taken together to form $-(CR^1R^2)_m-$;

R^5 is selected from the group consisting of H, hydroxy, alkyl, NH_2, $NHSO_2R^7$, $NHCOR^7$, and $NHCO_2R^7$;

15   R^7 is selected from the group consisting of alkyl; aryl; aralkyl; and alkyl optionally substituted with cycloalkyl, cycloalkenyl, heterocycle, alkenyl, alkynyl, alkoxyl, hydroxyl, halogen, aralkoxy, thioalkoxy, carboxy, alkoxycarbonyl, and carboxamide;

20   More preferred compounds are compounds M101-M112, M116, M117, M124, M125, M127-M129, M139, M140, M141-M150 in Figure-15.

In other embodiments, preferred compounds are represented by GB-2:

- 34 -

GB-2
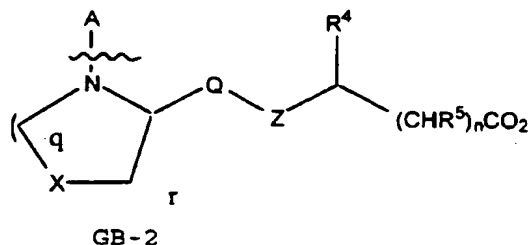
A is the same as defined in GB-1;

Q is -CH$_2$-, -CH=CH-, or -CH$_2$CH$_2$-;

5   Z is selected from the group consisting of: -CHR^4-, -CO-, O, S,
-SO-, -SO$_2$-, NR^4, NCOR^7, NSO$_2$R^7, -NCO$_2$R^7-, and -CONR^7-;

R^4 is selected from the group consisting of H, alkyl, aryl,
aralkyl; alkyl optionally substituted with cycloalkyl,
cycloalkenyl, heterocycle, alkenyl, alkynyl, alkoxyl, hydroxyl,

10 halogen, aralkoxy, thioalkoxy, carboxy, alkoxycarbonyl,
carboxamide, amine, alkylsulfone, or alkylsulfoxide;

R^5 is selected from the group consisting of H, hydroxy, alkyl,
NH$_2$, NHSO$_2$R^7, and NHCOR^7;

R^7 is selected from the group of alkyl, aryl, aralkyl; alkyl

15 optionally substituted with cycloalkyl, cycloalkenyl,
heterocycle, alkenyl, alkynyl, alkoxyl, hydroxyl, halogen,
aralkoxy, thioalkoxy, carboxy, alkoxycarbonyl, or carboxamide;

X is selected from the group consisting of -CH$_2$-, S, O, NR^4,
NCOR^7, and NSO$_2$R^7;

20 n =0-5;

q and r are independently selected from 1, 2.

More preferred compounds are in Figure 15, as are the most
preferred compounds M115, M118-M123, M126, M130-M135, in
figure-15.

- 35 -

The novel VLA-4 inhibitors claimed by Applicants map to the "Neg" feature of Model 1, and from 3-7 of features 1-7. . The compounds of the invention preferably have an inhibitory activity in the range of about 100 µM to about 0.5 nM,
5   preferably of less than about 50 µm, more preferably less than about 500 nM, and most preferably, less than about 50 nM.

Preferable inhibitors map to at least 3 of Features 1-7 of
10   Model 1, and have an IC_{50} value of less than about 50 nM. More preferable inhibitors map to at least 5 of Features 1-7.
ii)   Inhibitors which map to Model 2
In other embodiments the claimed VLA-4 inhibitors map to the "Neg" feature of Model 2, and at least four other features
15   selected from Features 1-8 of Model 2. More preferably, the inhibitors map to at least 6 features, and most preferably, at least 7 features, in addition to "Neg."
In most preferred embodiments, the claimed inhibitors which map to "Neg" and from 4 to 8 of Features 1-8 of Model 2,
20   have an IC_{50} value of about 50 µm to about 0.5 nM. The compounds of the invention preferably have an inhibitory activity in the range of about 100 µM to about 0.5 nM, preferably of less than about 50 µm, more preferably less than about 500 nM, and most preferably, less than about 50 nM.
25

The most preferable inhibitors map to at least 6 of the Feature of Model 2, and have an IC_{50} value of less than about 50 nM.
iii)   Inhibitors which map to Model 3

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- 36 -

        In yet other embodiments, the claimed compounds
comprise a scaffold which map to Model 3. Applicants have
discovered that compounds comprising those scaffolds have
excellent inhibitory activity ranging from about 500 μm to
5   about 0.5 nM. The compounds of the invention preferably have an
inhibitory activity in the range of about 100 μM to about 0.5
nM, preferably of less than about 50 μm, more preferably less
than about 500 nM, and most preferably, less than about 50 nM.

10   Those skilled in the art will recognize that inhibitors of
the invention may map to more than one of the claimed Models.

                        EXAMPLES

Example 1: Fit of claimed Models to the experimental structure
of a known VLA-4 Ligand

15   In order to further validate the claimed methods and
model, applicants determined whether known VLA-4 inhibitors fit
the claimed Model 1. Protein X-ray crystallography is a
powerful and commonly used experimental tool to provide
structural insight into the biological conformation of
20   macromolecules, and was used to determine the crystallographic
structure of vascular cell adhesion molecule 1 (VCAM ;
Brookhaven Code 1vca). This is a known physiological ligand of
the VLA-4 receptor, which contains the sequence Ile-Asp-Ser.
The Ile-Asp-Ser sequence is homologous to the Leu-Asp-Val
25   sequence from CS1 upon which the peptidomimetics used in the
claimed model were based. Peptides based upon Leu-Asp-Val
region have been shown to inhibit the VLA-4-VCAM interaction.
Thus, applicants hypothesized that VLA-4 antagonists, when they

                    SUBSTITUTE SHEET (RULE 26)

- 37 -

bind to VLA-4, may mimic the structure of the Ile-Asp-Ser
region of the VCAM structure.

The overlay between the model and Ile-Asp-Ser portion of
VCAM is shown in Figure 9.   As can be seen in Figure 9, the
5   Ile "maps" to the hydrophobic feature HYD3 and the carboxyl
group of the Asp "maps" to the negative ionizable feature NEG.
Additionally, the carbonyl of the Ile residue maps to the
hydrogen-bond acceptor feature HBA (Note that HBA describes
the feature HBA-1 and HBA-2). Thus, applicants were able to
10 confirm their claimed model by the mapping of the binding
epitope of a known ligand of VLA-4 to the Model-1.

Example 2 : How the Models Fit different chemical
templates

In order to further validate the claimed model 1, applicants
15 investigated its ability to fit known VLA-4 inhibitors which
were not used in the construction of the model, and were not
synthesized with any knowledge of the model. Model 1's ability
to map M2, M3 and M2 (Figure 3 b, c and d respectively) was
determined. The scaffolds of these three molecules are
20 structurally quite different, yet each maps to the claimed
model 1. Thus, applicants were able to confirm that the
claimed model 1 can, in fact, be used to identify VLA-4
inhibitors, and to identify alternative chemical
templates (Figure 10 ; see method 1c for definition of fit).

25 Example 3: Replacement of -Leu-Asp-Val- portion of known VLA-4
inhibitors with other scaffolds

Applicants used the claimed models and methods to design
alternative scaffolds to replace -LDV- portion of known VLA4
inhibitors. One such example is illustrated by M6 which fits

- 38 -

the models. This novel compound was determined to be a potent VLA4 inhibitor in a direct binding assay with an IC_{50} of 800 nM.

Example 4: Replacement of the DiphenylUrea Portion of known
5   VLA-4 Inhibitors

Applicants have used the claimed model 1 to identify mimetics of the diphenylurea portion of known VLA-4 inhibitors. The claimed model has two hydrophobic features (HYD2 and HYD5), which map to the two phenyl groups of the diphenylurea.
10   Based upon the claimed model it was known that these features and the HYD4 and HBD were arranged in a plane. Analysis of the X-ray structures of diphenylurea shows them to adopt a planar arrangement between the two phenyl rings(Figure 6). To further support the validity of the claimed model, data
15   was obtained which showed that replacement of the phenyl with a non-planar cyclohexyl group(M7 ; Figure 3g) (M8 ; Figure 3h) diminishes binding(M7 IC_{50} 10μM, M8 IC_{50} 50nM), thus confirming the importance of planarity.

Additionally, applicants have shown that shorter molecules
20   are weaker binders. Thus, for example, Phenylacetyl-Leu-Asp-Val-Pro (Figure 3i) has an IC_{50} of 2 μM, while oMePUPA-Leu-Asp-Val-Pro(Figure 3j) has an IC_{50} of 8nM, confirming that the diphenylurea is occupying a cavity in which the length and shape of the molecule lead to high affinity binding.

25   Applicants searched for molecules with similar shapes and volumes to the diphenylurea model (Figure 7) using the program catShape(Catalyst 3.1 Installation Guides and Release Notes, San Diego, Molecular Simulations Inc., 1996). Figure 8 shows examples of search hits.

SUBSTITUTE SHEET (RULE 26)

- 39 -

M11(Figure 3k) and M12(Figure 3l) were synthesized, the diphenylurea was replaced with the substructure returned from the search. M11 and M12 had an IC_{50} of 34 nM and 383 nM respectively.   Thus, the claimed invention provides

5   alternative caps which predict potent inhibitors of VLA-4.

Example 5: Replacement of the Leu-Asp-Val portion of known antagonists with scaffolds that can coordinate to a metal

The claimed invention teaches that desirable VLA-4 inhibitors are often involved in coordinating to a metal in the

10   VLA-4 receptor.   Therefore according to the claimed Model 3, scaffolds that can coordinate to the metal may be useful to replace the Leu-Asp-Val portion of VLA-4 antagonists. The evidence for the metal coordination of the VLA-4 antagonists includes structural and biological data.

15   i)All integrins require Mg^{2+} for ligand binding(Springer, TA, Nature, 346, 425-433).   This, together with the presence of Asp residues in the known VLA-4 receptor binding regions of Fibronectin(Leu-Asp-Val) and VCAM(Ile-Asp-Ser), suggested that the ligand coordinates to a metal.

20   ii)The applicants have discovered that the geometry of the hydrogen bond acceptor (HBA) and NEG feature in our model is consistent with the geometry of carboxyl groups and hydrogen-bond acceptors in molecules that coordinate to metals. For example, in Figure 12 the Mg^{2+} binding site of Leucine

25   Aminopeptidase is shown. In this site, the carbonyl oxygen of residue i and the carboxyl side chain of residue i+2 are coordinated to the Mg^{2+}. This geometry of the carbonyl and

- 40 -

carboxyl group is very similar to the geometry of the carbonyl group of Leu of residue i and the Asp carboxyl at i+2 in M2 (Figure 3b) when fitted to Model 1. In addition, the crystal structure of the Ile-Asp-Ser portion of VCAM, a known ligand to

5   VLA-4, shows that the geometry of the carbonyl oxygen of the Ile at position i and the carboxyl of the Asp at i+2 are similar to the geometry of known metal binding proteins (see Figure 12) and also to model 1.

Applicants searched for scaffolds that could coordinate to

10 a metal, using Model 3. In Figure 13 they show how M13 (Figure 3m) and M14 (Figure 3n) fit the Model 3.
Applicants also searched chemical databases for other molecules that fit Model 3. Figure 14 shows two compounds that contain scaffolds that are commercially available and map to Model 3.

15   It will be apparent to those skilled in the art that various modifications and variations can be made in the claimed invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention encompass modifications and variations of the invention

20 provided that they come within the scope of the appended claims and their equivalents.

- 41 -

WHAT IS CLAIMED IS:

1. A three dimensional pharmacophore model of a compound
having VLA-4 inhibitory activity, said model comprises NEG
("N")

	Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
N	NEG	-8.564	1.564	-0.236	1.702

5 and at least three features selected from the group consisting
of

	Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
1	HBA-1	-1.276	-1.259	-1.47	1.702
	HBA-2	-2.323	1.539	-1.35	1.702
2	HBD-1	6.693	1.988	-0.168	1.702
	HBD-2	7.217	0.939	2.630	2.302
3	HYD2	2.777	-1.061	-1.1501	1.702
4	HYD3	-3.803	-4.061	0.270	1.702
5	HYD4	9.377	2.219	1.050	1.702
6	HYD5	8.677	4.439	-1.330	1.702
7	HYD6	-9.123	-1.501	1.110	1.702

2. The model of claim 1 wherein the model comprises at least
five features selected from the group consisting of Feature 1,

- 42 -

Feature 2, Feature 3, Feature 4, Feature 5, Feature 6 and
Feature 7.

3.   The model of claim 2 wherein the model comprises at least
seist seven features selected from the group consisting of Feature 1,
5   Feature 2, Feature 3, Feature 4, Feature 5, Feature 6 and
Feature 7.

4.   A compound which fits the model of claim 1, said compound
having an IC_{50} value of less than about 100 uM in a VLA-4
direct binding assay.

10   5.   The compound of claim 4 wherein said compound has an IC_{50}
of less than about 50 μM.

6.   The compound of claim 5 wherein said compound has an IC_{50}
of less than about 1 μM.

7.   The compound of claim 6 wherein said compound has an IC_{50}
15   of less than about 500 nM.

8.   The compound of claim 7 wherein said compound has an IC_{50}
of less than about 100 nM.

9.   The compound of claim 8 wherein said compound has an IC_{50}
of less than about 50 nM.

20   10.   A compound which fits the model of claim 2, said
compound having an IC_{50} value of less than about 100 μM in a
VLA-4 direct binding assay.
11.   The compound of claim 10 wherein said compound has an IC_{50}
of less than about 50 μM.

SUBSTITUTE SHEET (RULE 26)

- 43 -

12. The compound of claim 11 wherein said compound has an IC_{50} of less than about 1 μM.

13. The compound of claim 12 wherein said compound has an IC_{50} of less than about 500 nM.

14. The compound of claim 13 wherein said compound has an IC_{50} of less than about 100 nM.

15. The compound of claim 14 wherein said compound has an IC_{50} of less than about 50 nM.

16. A compound which fits the model of claim 3 having an IC_{50} value of less than about 100 μM in a VLA-4 direct binding assay.

17. The compound of claim 16 wherein said compound has an IC_{50} of less than about 50 μM.

18. The compound of claim 17 wherein said compound has an IC_{50} of less than about 1 μM.

19. The compound of claim 18 wherein said compound has an IC_{50} of less than about 500 nM.

20. The compound of claim 19 wherein said compound has an IC_{50} of less than about 100 nM.

21. The compound of claim 20 wherein said compound has an IC_{50} of less than about 50 nM.

- 44 -

22.   A method for identifying a chemical compound having an IC$_{50}$ value of less than about 100 µM in a VLA-4 direct binding assay, said method comprising the steps of:

    a) selecting an experimental compound structure to be
5   evaluated for VLA-4 activity;

    b) computationally or experimentally obtaining a three dimensional structure of said experimental compound;

    c) evaluating whether the three dimensional structure of said experimental compound fits the pharmacophore model of
10 claim 1; and

    d) determining whether said experimental compound has an IC$_{50}$ value of less than about 100 µM in a VLA-4 direct binding assay.

23.   A compound having an IC$_{50}$ of less than about 50 µM
15 identified by the method of claim 22.

24.   The compound of claim 23 wherein said compound has an IC$_{50}$ of less than about 1 µM.

25.   The compound of claim 24 wherein said compound has an IC$_{50}$ of less than about 500 nM.

20 26.   The compound of claim 25 wherein said compound has an IC$_{50}$ of less than about 100 nM.

27.   The compound of claim 26 wherein said compound has an IC$_{50}$ of less than about 50 nM.

SUBSTITUTE SHEET (RULE 26)

- 45 -

28.   A three dimensional pharmacophore model of a compound having VLA-4 inhibitory activity, said model comprises NEG ("N") and at least four features selected from the group consisting of features 1 to 8

	Feature	x Å	y Å	z Å	tolerance Å
N	NEG	5.19	2.48	-0.84	1.5
1	HBA1-1	2.625	0.078	-0.451	1.5
	HBA1-2	1.434	2.840	-0.448	1.5
2	HBA2-1	6.038	-1.968	-0.039	1.5
	HBA2-2	8.314	-2.560	1.832	1.5
3	HBD-1	-6.17	-0.82	0.767	1.5
	HBD-2	-6.606	-3.3	2.412	1.5
4	HYD2	-1.126	-0.54	1.532	1.5
5	HYD3	1.054	-3.780	-2.528	1.5
6	HYD4	-8.786	-1.3	1.972	1.5
7	HYD5	-8.786	-0.580	-0.788	1.5
8	HYD6	8.594	2.12	-3.428	1.5

5   29.   The model of claim 28 wherein the model comprises at least five features selected from the group consisting of Feature 1, Feature 2, Feature 3, Feature 4, Feature 5, Feature 6, Feature 7 and Feature 8.

30.   The model of claim 29 wherein the model comprises at least 10   seven features selected from the group consisting of Feature 1, Feature 2, Feature 3, Feature 4, Feature 5, Feature 6, Feature 7 and Feature 8.

- 46 -

31. A compound which fits the model of claim 28 and has an IC$_{50}$ value of less than about 100 μM in a VLA-4 direct binding assay.

32. The compound of claim 31 wherein said compound has an IC$_{50}$ of less than about 50μM.

33. The compound of claim 32 wherein said compound has an IC$_{50}$ of less than about 1μM.

34. The compound of claim 33 wherein said compound has an IC$_{50}$ of less than about 500 nM.

35. The compound of claim 34 wherein said compound has an IC$_{50}$ of less than about 100 nM.

36. The compound of claim 35 wherein said compound has an IC$_{50}$ of less than about 50 nM.

37. A compound which fits the model of claim 29 and has an IC$_{50}$ value of less than about 100 μM in a VLA-4 direct binding assay

38. The compound of claim 37 wherein said compound has an IC$_{50}$ of less than about 50 μM.

39. The compound of claim 38 wherein said compound has an IC$_{50}$ of less than about 1 μM.

40. The compound of claim 39 wherein said compound has an IC$_{50}$ of less than about 500 nM.

SUBSTITUTE SHEET (RULE 26)

- 47 -

41.   The compound of claim 40 wherein said compound has an IC_{50} of less than about 100 nM.

42.   The compound of claim 41 wherein said compound has an IC_{50} of less than about 50 nM.

5   43.   A compound which fits the model of claim 30 and has an IC_{50} value of less than about 100 μM in a VLA-4 direct binding assay.

44.   The compound of claim 43 wherein said compound has an IC_{50} of less than about 50 μM.

10   45.   The compound of claim 44 wherein said compound has an IC_{50} of less than about 1μM.

46.   The compound of claim 45 wherein said compound has an IC_{50} of less than about 500 nM.

47.   The compound of claim 46 wherein said compound has an IC_{50}
15   of less than about 100 nM.

48.   The compound of claim 47 wherein said compound has an IC_{50} of less than about 50 nM.

49.   A method for identifying a chemical compound having an IC_{50} value of less than about 100 μM in a VLA-4 direct binding
20   assay, said method comprising the steps of:
        a) selecting an experimental compound to be evaluated for VLA-4 activity;
        b) obtaining a three dimensional structure of said experimental compound;

- 48 -

c) evaluating whether the three dimensional structure of said experimental compound fits the model of claim 28; and

d) determining whether said experimental compound has an IC_{50} value of less than about 100 µM in a VLA-4 direct binding

5   assay.

50. A compound having an IC_{50} of less than about 50 µM identified by the method of claim 49.

51. The compound of claim 50 wherein said compound has an IC_{50} of less than about 1 µM.

10 52. The compound of claim 51 wherein said compound has an IC_{50} of less than about 500 nM.

53.   The compound of claim 52 wherein said compound has an IC_{50} of less than about 100 nM.

54.   The compound of claim 53 wherein said compound has an

15 IC_{50} of less than about 50 nM.

- 49 -

55. A three dimensional pharmacophore model of a compound having VLA-4 inhibitory activity, said model comprising:

Feature	x (Å)	y (Å)	z (Å)	tolerance (Å)
Carboxyl C	-3.131	-2.023	2.824	1.2
Carboxyl O1	-3.513	-0.027	4.108	0.9
Carboxyl O2	-1.487	-0.895	4.167	0.9
Carbonyl C	-2.241	2.730	0.315	0.9
Carbonyl O	-3.067	3.241	1.064	0.9

56.   A method for identifying a chemical compound having an IC_{50} value of less than about 100 µM in a VLA-4 direct binding assay, said method comprising the steps of:

a) selecting an experimental compound structure to be evaluated for VLA-4 activity;

b) computationally or experimentally obtaining a three dimensional structure of said experimental compound;

c) evaluating whether the three dimensional structure of said experimental compound fits the model of claim 55; and

d) determining whether said experimental compound has an IC_{50} value of less than about 100 µM in a VLA-4 direct binding assay.

57.   A compound having an IC_{50} of less than about 50 µM identified by the method of claim 56.

SUBSTITUTE SHEET (RULE 26)

- 50 -

5   58.   The compound of claim 57 wherein said compound has an IC_{50} of less than about 1 μM.

59.   The compound of claim 58 wherein said compound has an IC_{50} of less than about 500 nM.

60.   The compound of claim 59 wherein said compound has an
10 IC_{50} of less than about 100 nM.

61.   The compound of claim 60 wherein said compound has an IC_{50} of less than about 50 nM.

62.   A method for identifying a chemical compound having VLA-4 inhibitory activity, said method comprising the steps of:
15   a) selecting an experimental compound structure to be evaluated for VLA-4 inhibitory activity;

        b) determining whether said experimental compound comprises a substructure having about the same shape and volume as diphenylurea;

20   c) determining the three-dimensional structure of said experimental compound;

        d) mapping the three-dimensional structure of said experimental compound to Neg and Feature 1 of the model of claim 1;

25   e) determining if said substructure is within about 0.5 to about 3.0 Å of any one of Features 2, 3, 5 or 6 of the VLA-4 model of claim 1.

63.   The method of claim 62 further comprising the step of determining whether the experimental compound has an IC_{50} of
30 less than about 100 μM in a VLA-4 direct binding assay.

64. The method of claim 63 further comprising the step of determining whether the experimental compound has an IC_{50} of less than about 50 μM in a VLA-4 direct binding assay.

65. The method of claim 64 further comprising the step of determining whether the experimental compound has an IC_{50} of less than about 1 μM in a VLA-4 direct binding assay.

66. The method of claim 65 further comprising the step of determining whether the experimental compound has an IC_{50} of less than about 500 nM in a VLA-4 direct binding assay.

67. The method of claim 66 further comprising the step of determining whether the experimental compound has an IC_{50} of less than about 100 nM in a VLA-4 direct binding assay.

68. The method of claim 67 further comprising the step of determining whether the experimental compound has an IC_{50} of less than about 50 nM in a VLA-4 direct binding assay.

69. Cell adhesion inhibitors comprising Formula GB-1
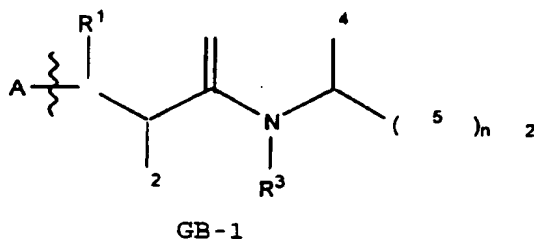
GB-1

A is selected from the group consisting of alkyl; aliphatic acyl optionally substituted with N-alkyl- or N-arylamido;

- 52 -

5   aroyl; heterocycloyl; alkyl- or arylsulfonyl; aralkylcarbonyl
     optionally substituted with aryl; heterocycloalkylcarbonyl;
     alkoxycarbonyl; aralkyloxycarbonyl; cycloalkylcarbonyl
     optionally fused with aryl; heterocycloalkoxycarbonyl;
     alkylaminocarbonyl; arylamino carbonyl and aralkylaminocarbonyl
10   optionally substituted with bis(alkylsulfonyl)amino,
     alkoxycarbonylamino or alkenyl; alkylsulfonyl; aralkylsulfonyl;
     arylsulfonyl; cycloalkylsulfonyl optionally fused with aryl;
     heterocyclylsulfonyl; heterocyclylalkylsulfonyl;
     aralkoxycarbonyl; aryloxycarbonyl; cycloalkyloxycarbonyl;
15   heterocyclyloxycarbonyl; heterocyclylalkoxycarbonyl; mono- or
     di-alkylaminocarbonyl optionally substituted with aryl;
     (alkyl)(aralkyl)aminocarbonyl; mono- or di-
     aralkylaminocarbonyl; mono- or di-arylaminocarbonyl;
     (aryl)(alkyl)aminocarbonyl; mono- or di-
20   cycloalkylaminocarbonyl; heterocyclylaminocarbonyl;
     heterocyclylalkylaminocarbonyl;
     (alkyl)(heterocyclyl)aminocarbonyl;
     (alkyl)(heterocyclylalkyl)aminocarbonyl;
     (aralkyl)(heterocyclyl)aminocarbonyl;
25   (aralkyl)(heterocyclylalkyl)aminocarbonyl; alkenoyl optionally
     substituted with aryl; alkenylsulfonyl optionally substituted
     with aryl; alkynoyl optionally substituted with aryl;
     alkynylsulfonyl optionally substituted with aryl;
     cycloalkenylcarbonyl; cycloalkenylsulfonyl; cycloalkylalkanoyl;
30   cycloalkylalkylsulfonyl; arylaroyl, biarylsulfonyl;
     alkoxysulfonyl; aralkoxysulfonyl; alkylaminosulfonyl;
     aryloxysulfonyl; arylaminosulfonyl; N-arylurea-substituted
     alkanoyl; N-arylurea-substituted alkylsulfonyl; cycloalkenyl-
     substituted carbonyl; cycloalkenyl-substituted sulfonyl;
35   alkenoxycarbonyl optionally substituted with aryl;

SUBSTITUTE SHEET (RULE 26)

- 53 -

5   alkenoxysulfonyl optionally substituted with aryl;
     alkynoxycarbonyl optionally substituted with aryl;
     alkynoxysulfonyl optionally substituted with aryl; alkenyl- or
     alkynyl-aminocarbonyl optionally substituted with aryl;
     alkenyl- or alkynyl-aminosulfonyl optionally substituted with
10   aryl; acylamino-substituted alkanoyl; acylamino-substituted
     alkylsulfonyl; aminocarbonyl-substituted alkanoyl; carbamoyl-
     substituted alkanoyl; carbamoyl-substituted alkylsulfonyl;
     heterocyclylalkanoyl; heterocyclylaminosulfonyl; carboxyalkyl-
     substituted aralkoyl; carboxyalkyl-substituted aralkylsulfonyl;
15   oxocarbocyclyl-fused aroyl; oxocarbocyclyl-fused arylsulfonyl;
     heterocyclylalkanoyl; N',N'-alkyl, arylhydrazinocarbonyl;
     aryloxy-substituted alkanoyl and heterocyclylalkylsulfonyl;
     alkenyl, alkynyl, cycloalkyl, aryl-fused cycloalkyl,
     cycloalkenyl, aryl, aryl-substituted alkyl ("aralkyl"), aryl-
20   substituted alkenyl or alkynyl, cycloalkyl-substituted alkyl,
     cycloalkenyl-substituted cycloalkyl, biaryl, alkoxy, alkenoxy,
     alkynoxy, aryl-substituted alkoxy ("aralkoxy"), aryl-
     substituted alkenoxy or alkynoxy, alkylamino, alkenylamino or
     alkynylamino, aryl-substituted alkylamino, aryl-substituted
25   alkenylamino or alkynylamino, aryloxy, arylamino, N-alkylurea-
     substituted alkyl, N-arylurea-substituted alkyl,
     alkylcarbonylamino-substituted alkyl, aminocarbonyl-substituted
     alkyl, heterocyclyl, heterocyclyl-substituted alkyl,
     heterocyclyl-substituted amino, carboxyalkyl substituted
30   aralkyl, oxocarbocyclyl-fused aryl and heterocyclylalkyl;

     n=1-4;
     When R^3 is H, n=2-4; or when n=1, only R^3 or R^5 is H;
     R^1 and R^4 are independently selected from the group consisting
     of H, alkyl, aryl, aralkyl; alkyl optionally substituted with

SUBSTITUTE SHEET (RULE 26)

- 54 -

5   cycloalkyl, cycloalkenyl, heterocycle, alkenyl, alkynyl,
    alkoxyl, hydroxyl, halogen, aralkoxy, thioalkoxy, carboxy,
    alkoxycarbonyl, carboxamide, amine, alkylsulfone, and
    alkylsulfoxide;

    R^2 is selected from the group consisting of H, alkyl: alkyl
10 optionally substituted with amine, cycloalkyl, alkylsulfone,
    and alkylsulfoxide;

    R^3 is selected from the group consisting of H, alkyl, and alkyl
    optionally substituted with aralkoxy, hydroxy;

    X is selected from the group consisting of $-CH_2-$, S, O, NR^4,
15 $NCOR^7$, and NSO_2R^7;

    m is 3 or 4;

    p is 3 or 4;

    q and r are independently 1 or 2;

    R^1 and R^2 may be taken together to form $-(CR^1R^2)_p-$, or -
20 $(CR^1R^2)_qX(CR^1R^2)_r-$;

    R^3 and R^4 may be taken together to form $-(CR^1R^2)_m-$ or   -
    $(CR^1R^2)_qX(CR^1R^2)_r-$;

    R^3 and R^5 may be taken together to form $-(CR^1R^2)_m-$;

    R^5 is selected from the group consisting of H, hydroxy,
25 alkyl, NH_2, $NHSO_2R^7$, $NHCOR^7$, and $NHCO_2R^7$;

    R^7 is selected from the group consisting of alkyl; aryl;
    aralkyl; and alkyl optionally substituted with cycloalkyl,
    cycloalkenyl, heterocycle, alkenyl, alkynyl, alkoxyl, hydroxyl,
    halogen, aralkoxy, thioalkoxy, carboxy, alkoxycarbonyl, and
30 carboxamide;

    70. Cell adhesion inhibitors comprising Formula GB-2

SUBSTITUTE SHEET (RULE 26)

- 55 -

GB-2
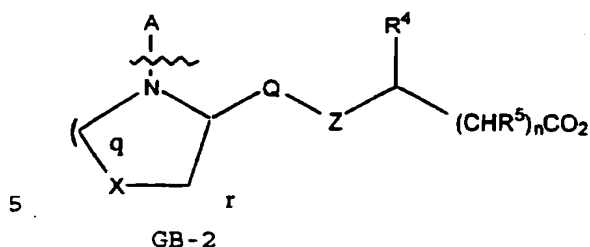
A is the same as defined in GB-1;

Q is $-CH_2-$, $-CH=CH-$, or $-CH_2CH_2-$;

Z is selected from the group consisting of: $-CHR^4-$, $-CO-$, O, S, $-SO-$, $-SO_2-$, NR^4 , $NCOR^7$, NSO_2R^7 , $-NCO_2R^7-$, and $-CONR^7-$;

R^4 is selected from the group consisting of H, alkyl, aryl, aralkyl; alkyl optionally substituted with cycloalkyl, cycloalkenyl, heterocycle, alkenyl, alkynyl, alkoxyl, hydroxyl, halogen, aralkoxy, thioalkoxy, carboxy, alkoxycarbonyl, carboxamide, amine, alkylsulfone, or alkylsulfoxide;

R^5 is selected from the group consisting of H, hydroxy, alkyl, NH_2 , $NHSO_2R^7$, and $NHCOR^7$;

R^7 is selected from the group of alkyl, aryl, aralkyl; alkyl optionally substituted with cycloalkyl, cycloalkenyl, heterocycle, alkenyl, alkynyl, alkoxyl, hydroxyl, halogen, aralkoxy, thioalkoxy, carboxy, alkoxycarbonyl, or carboxamide;

X is selected from the group consisting of $-CH_2-$, S, O, NR^4 , $NCOR^7$, and NSO_2R^7 ;

n = 0-5;

q and r are independently selected from 1, 2.

SUBSTITUTE SHEET (RULE 26)

1/23

FIG. 1a

FIG. 1b

FIG. 1c

FIG 2

FIG. 3a   M1

FIG. 3b   M2

FIG. 3c   M3

FIG. 3d   M4

FIG. 3e M5

FIG. 3f M6

FIG. 3g M7

FIG. 3h M8

FIG. 3i M9

FIG. 3j M10

SUBSTITUTE SHEET (RULE 26)

FIG. 3k   M11

FIG. 3l   M12

FIG. 3m   M13

FIG. 3n   M14

FIG. 3o   M15

FIG. 3p M16

FIG. 3q M17

FIG. 3r M18

FIG. 3s M19

FIG. 3t M20

FIG. 3u M21

FIG. 3v M22
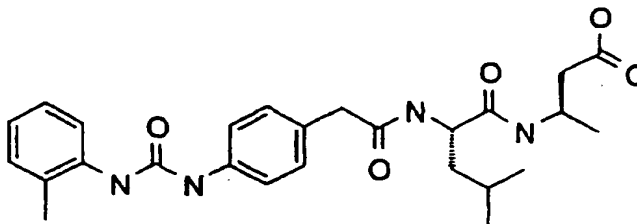
FIG. 3w M23

FIG. 3x M24

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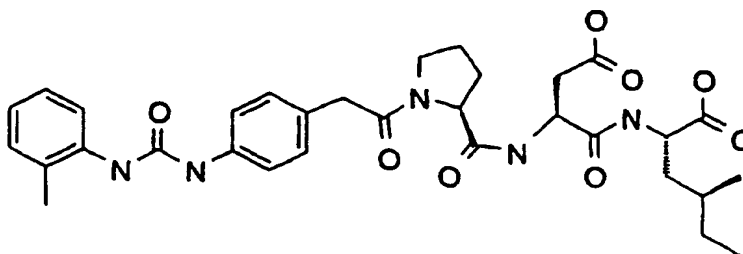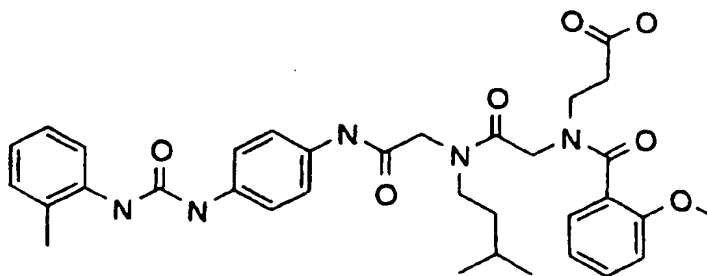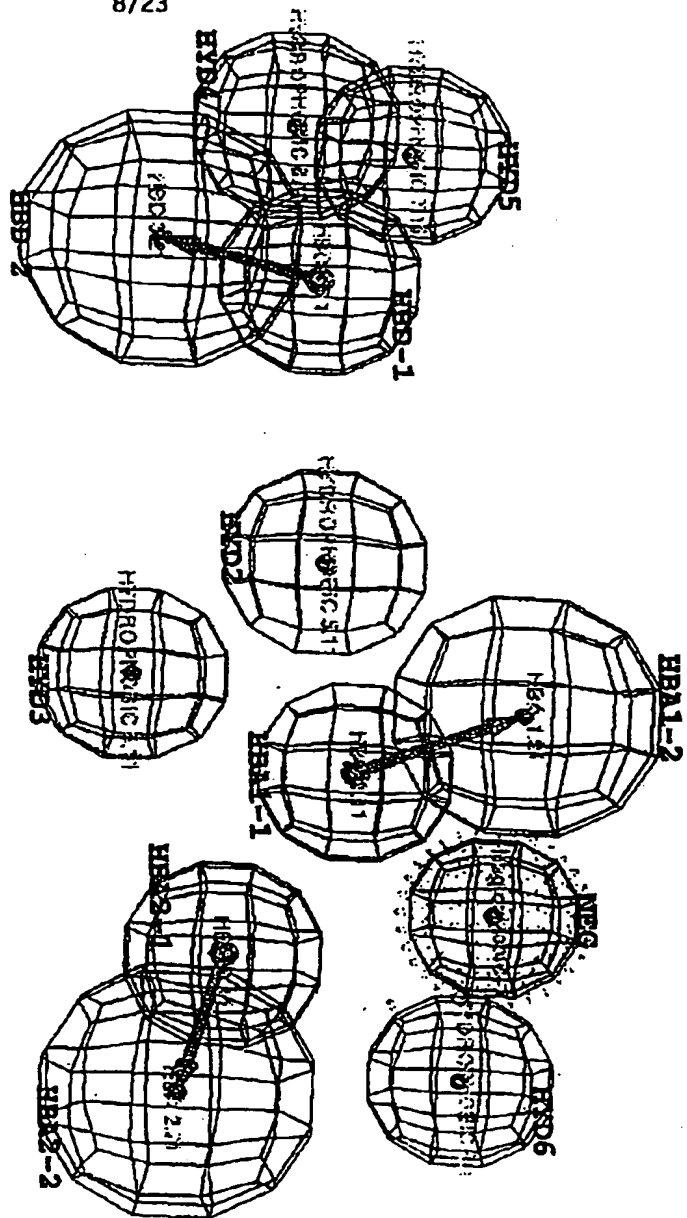
FIG4A
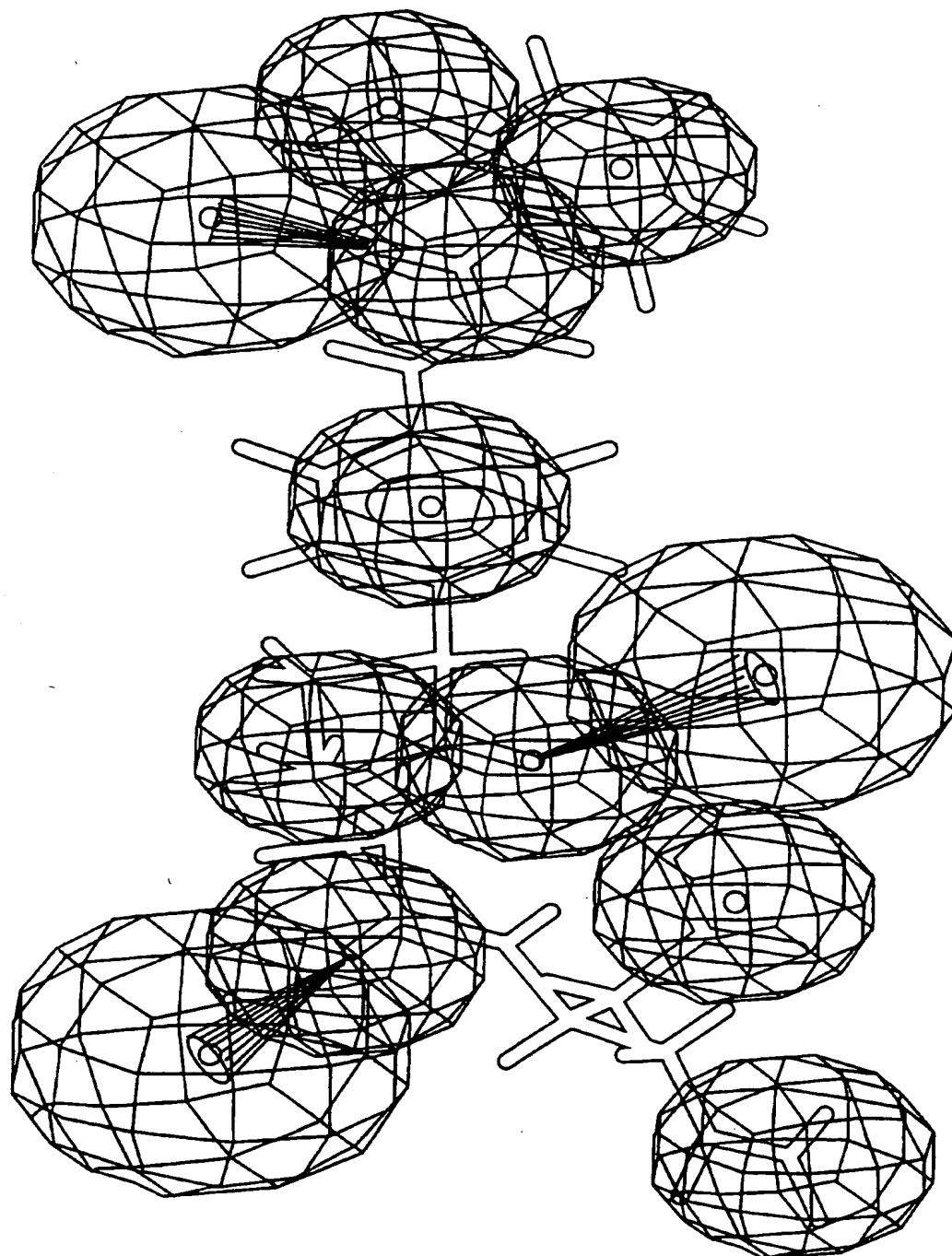
FIG. 4B

QIDSP LOOP IN VACM
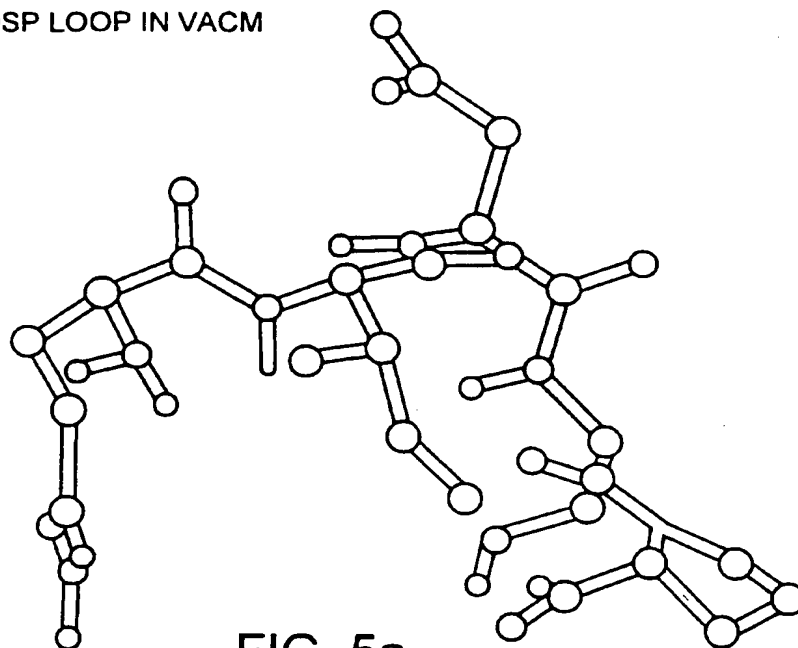
FIG. 5a

HYPOTHESIS BASED
ON C=0 OF Q38
AND CO2 OF D40
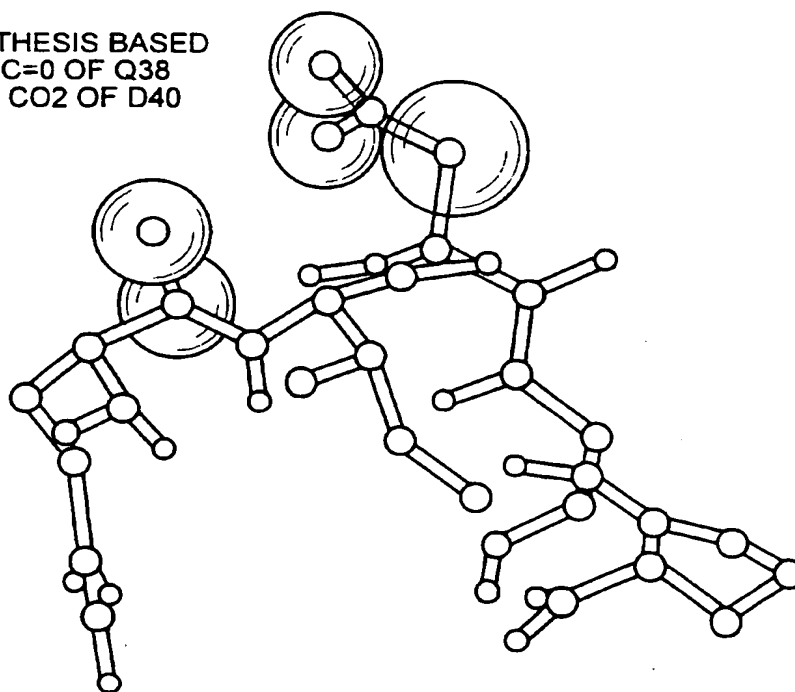
FIG. 5b

FIG. 6
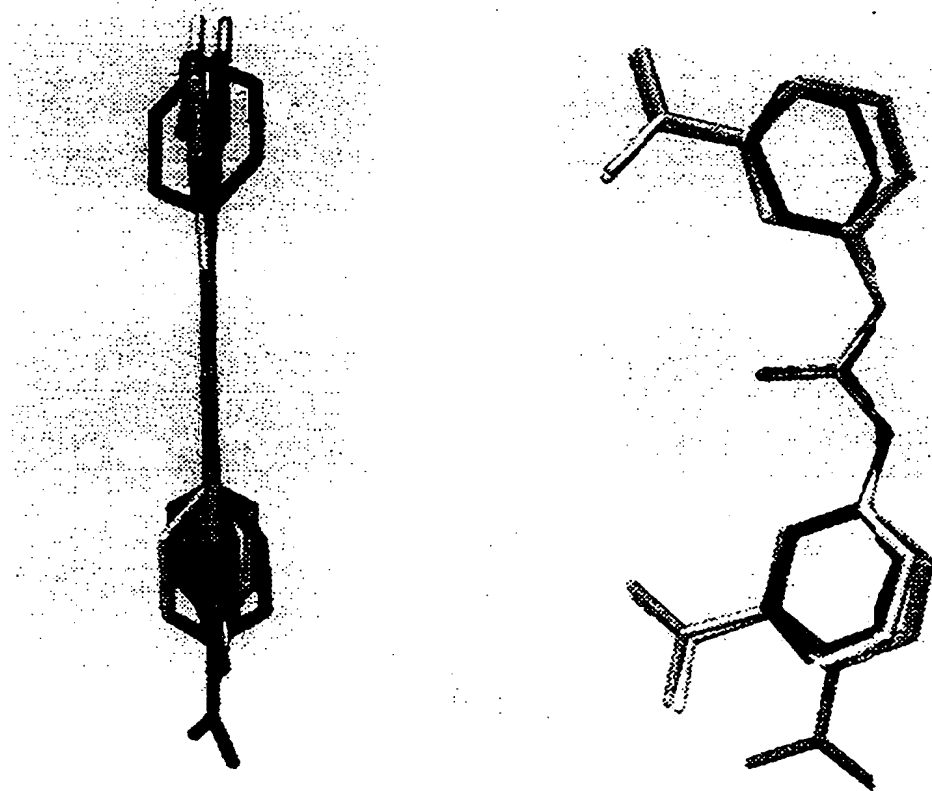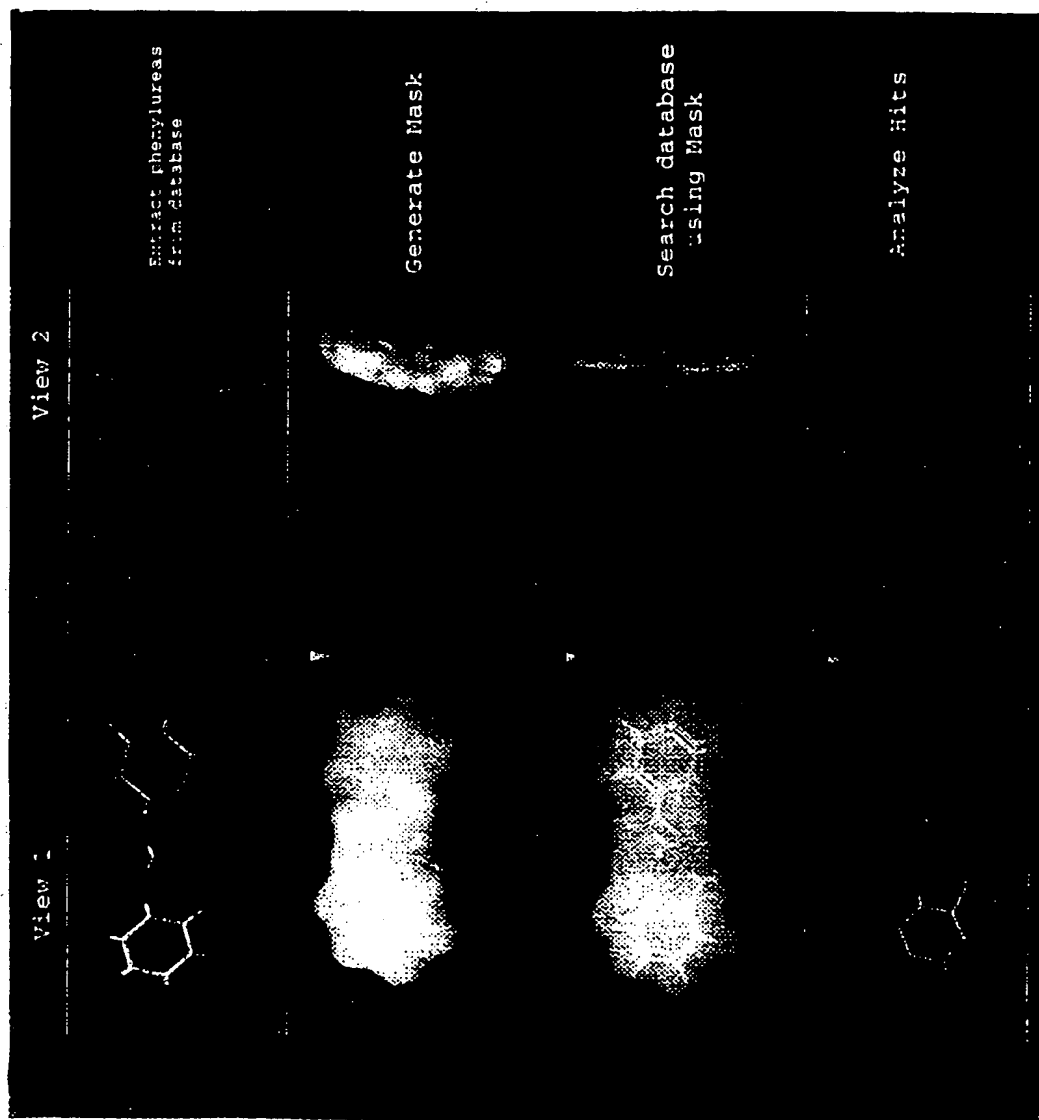
FIG. 7
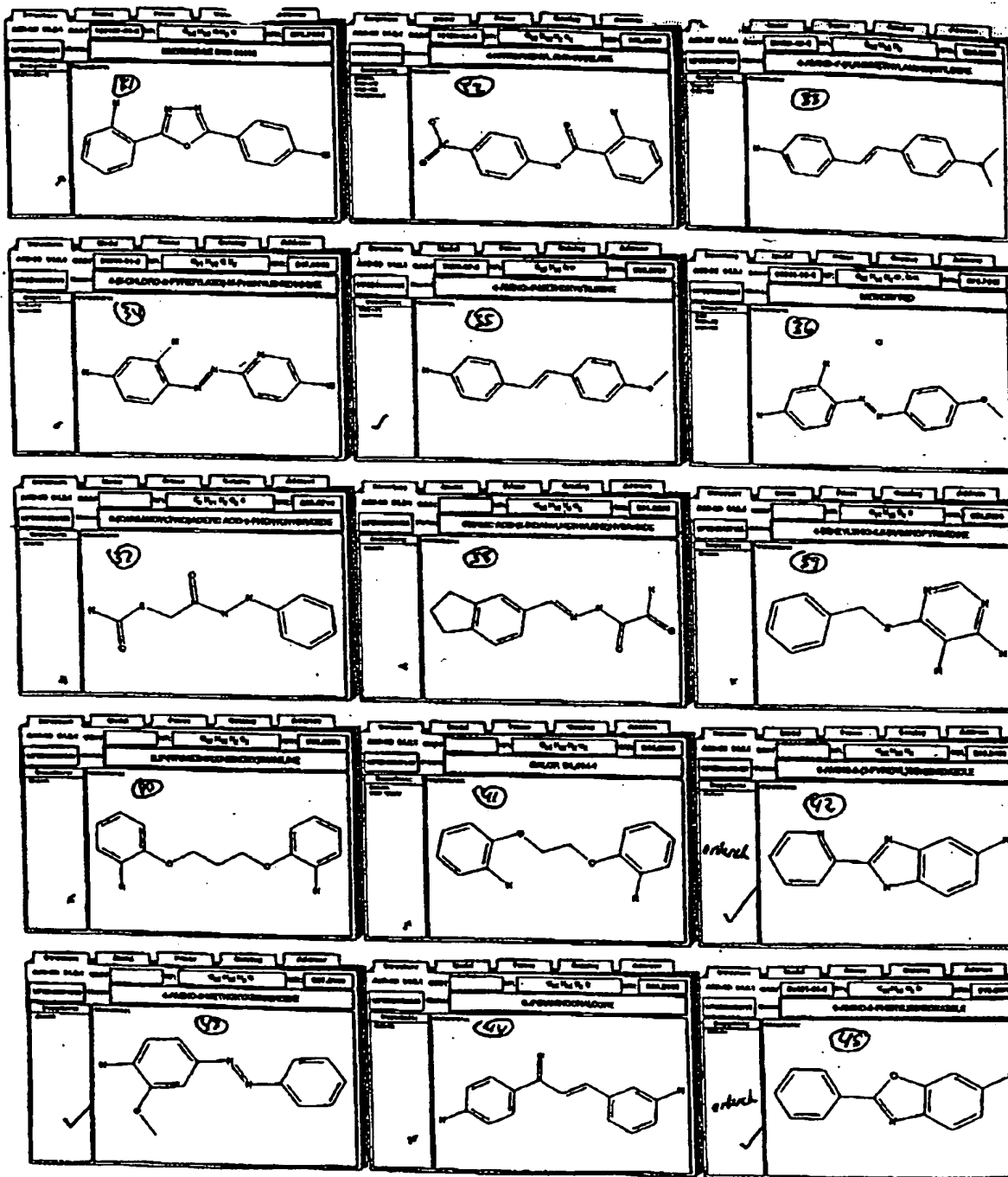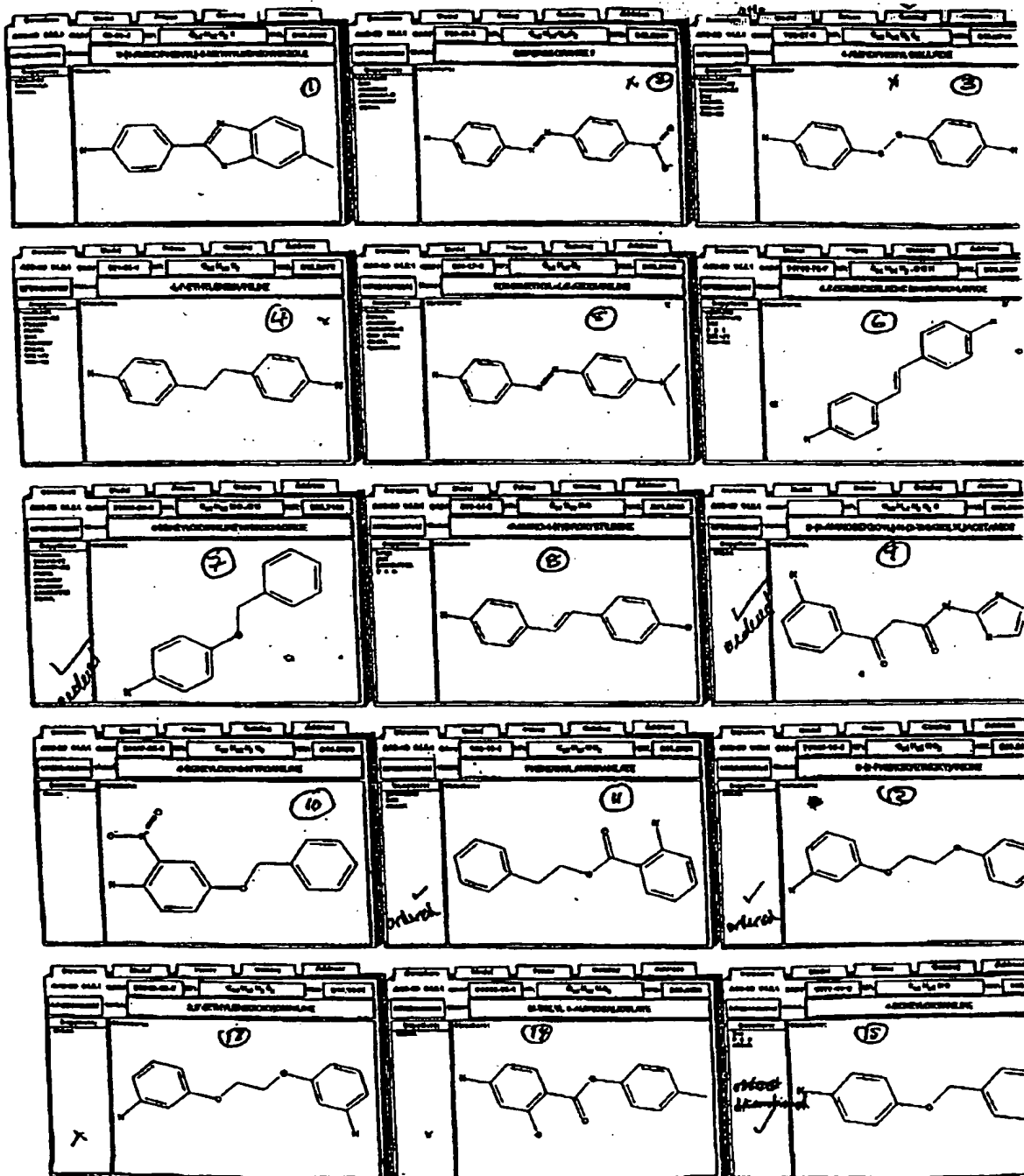
FIG 8

FIG 8

FIT OF THE X-RAY OF VCAM (38-42) TO THE VLA4 MODEL
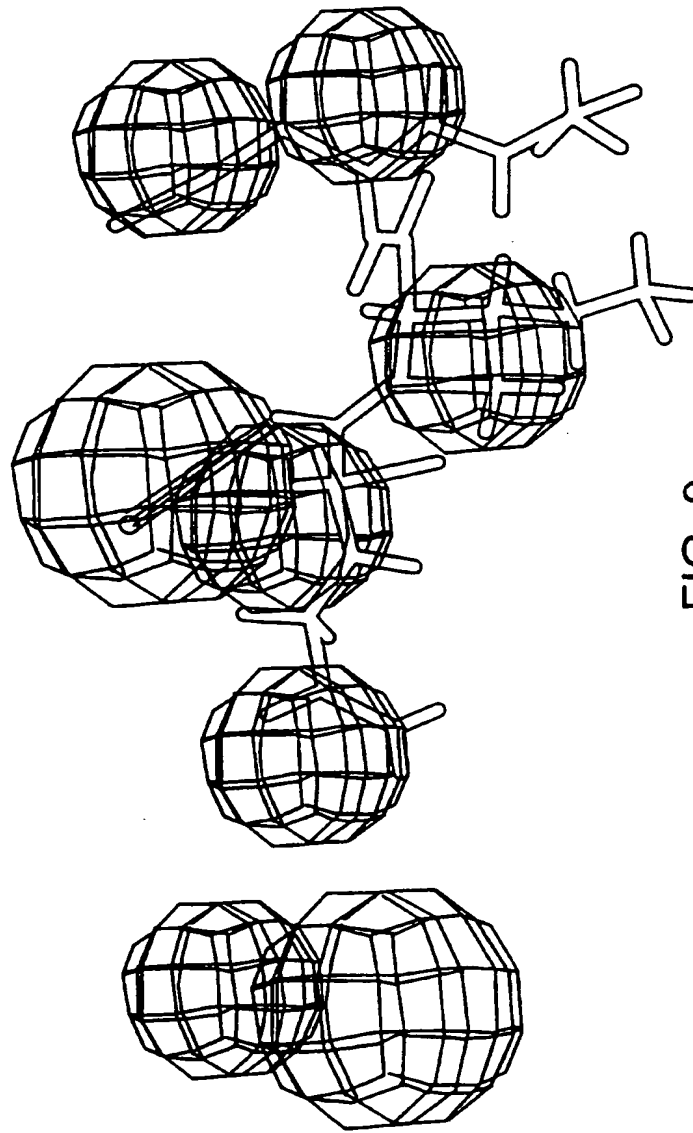
FIG. 9
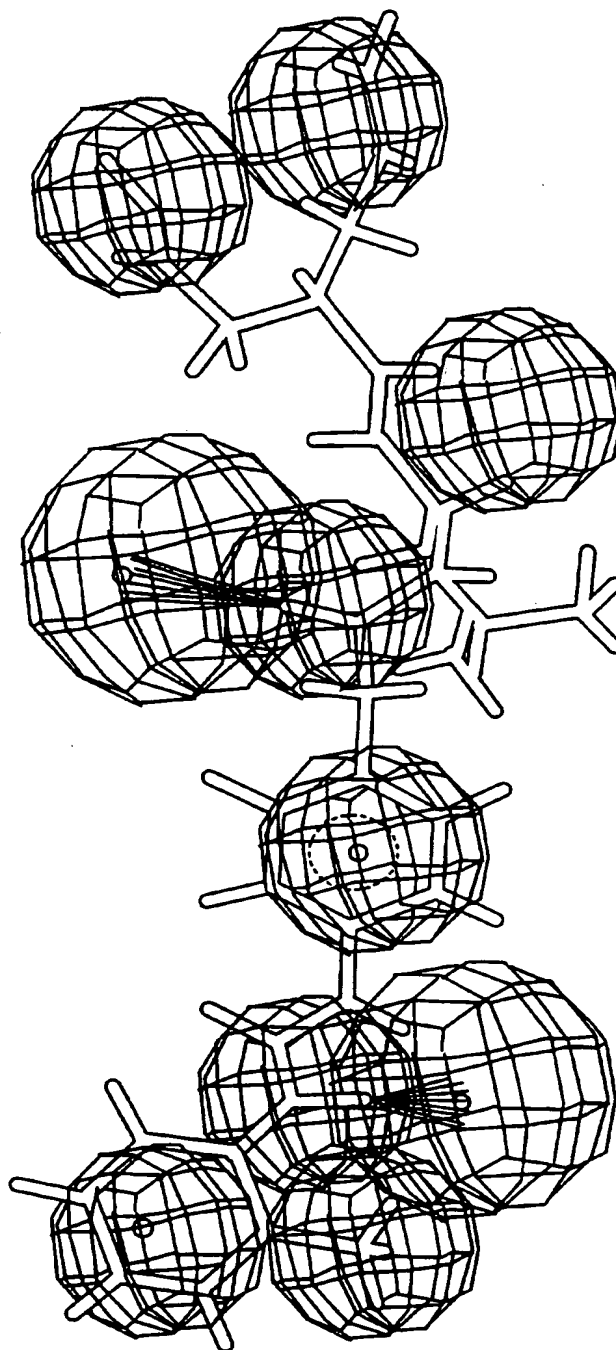
FIG. 10

FIG. 11A
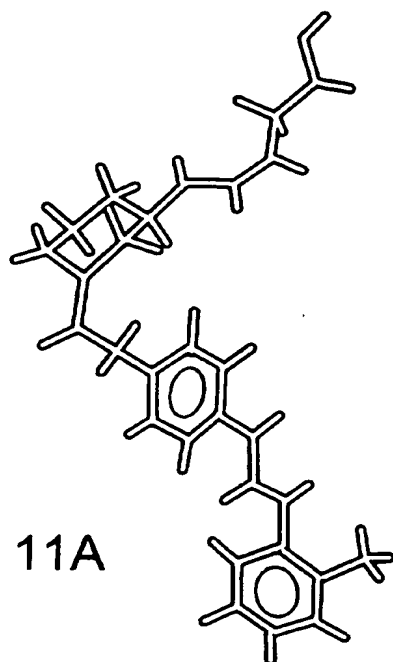
FIG. 11B
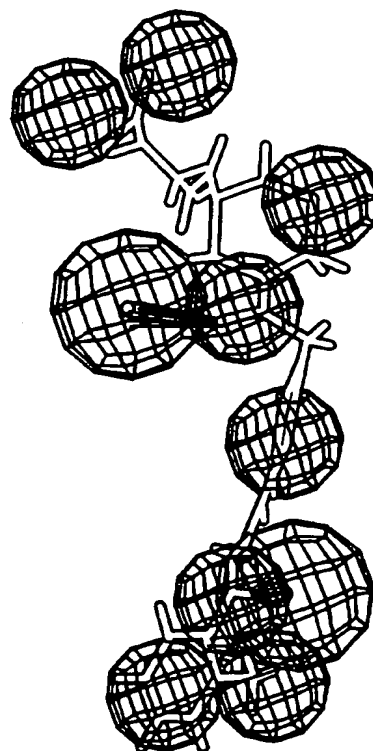
FIG. 12
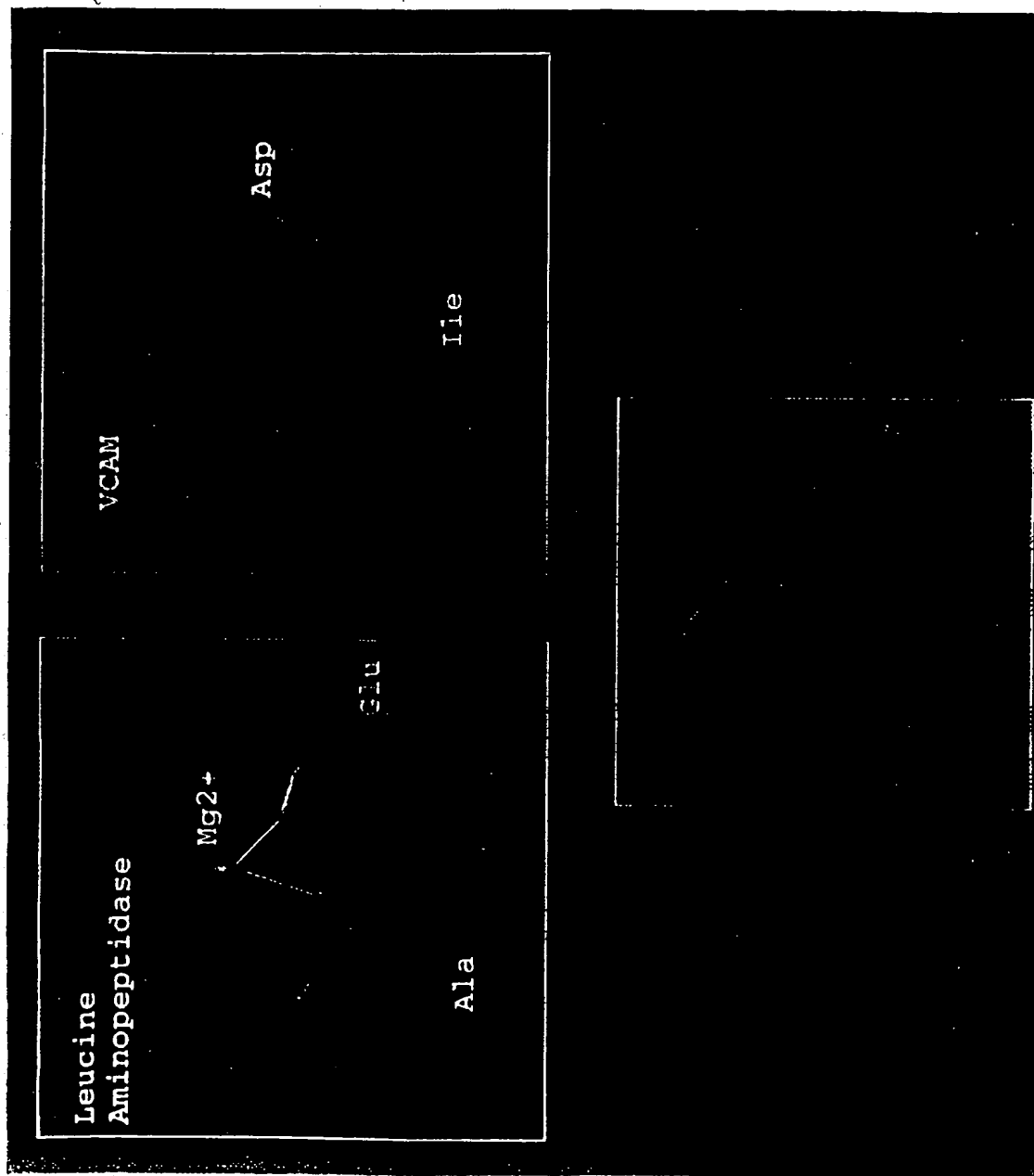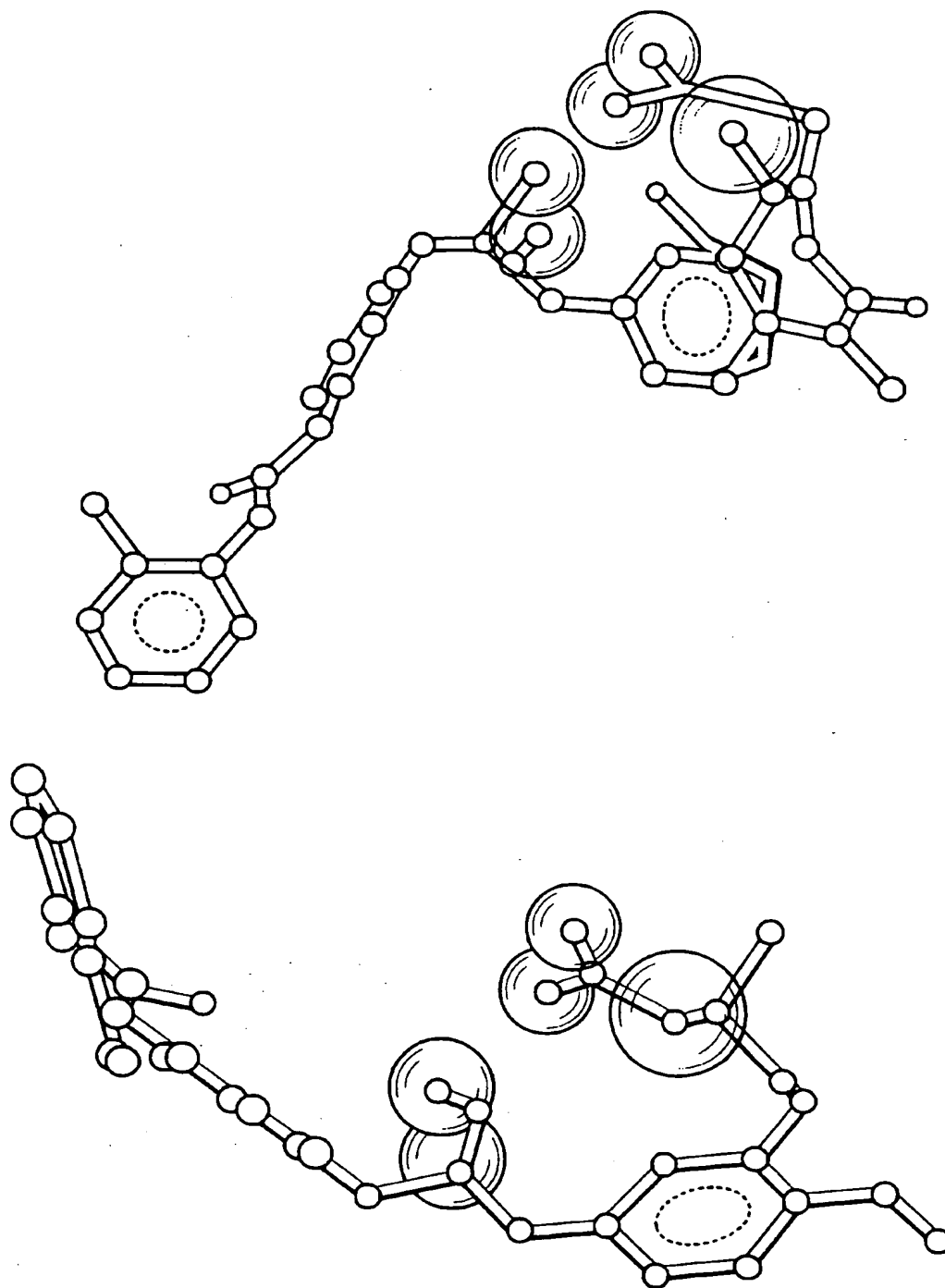
FIG 13

FIG. 14

Figure 15

50 Compounds

M101	M102	M103	M104
M105	M106	M107	M108
M109	M110	M111	M112
M113	M114	M115	M116
M117	M118	M119	M120
M121	M122	M123	M124

Figure 15

50 Compounds

M125	M126	M127	M128
M129	M130	M131	M132
M133	M134	M135	M136
M137	M138	M139	M140
M141	M142	M143	M144
M145	M146	M147	M148
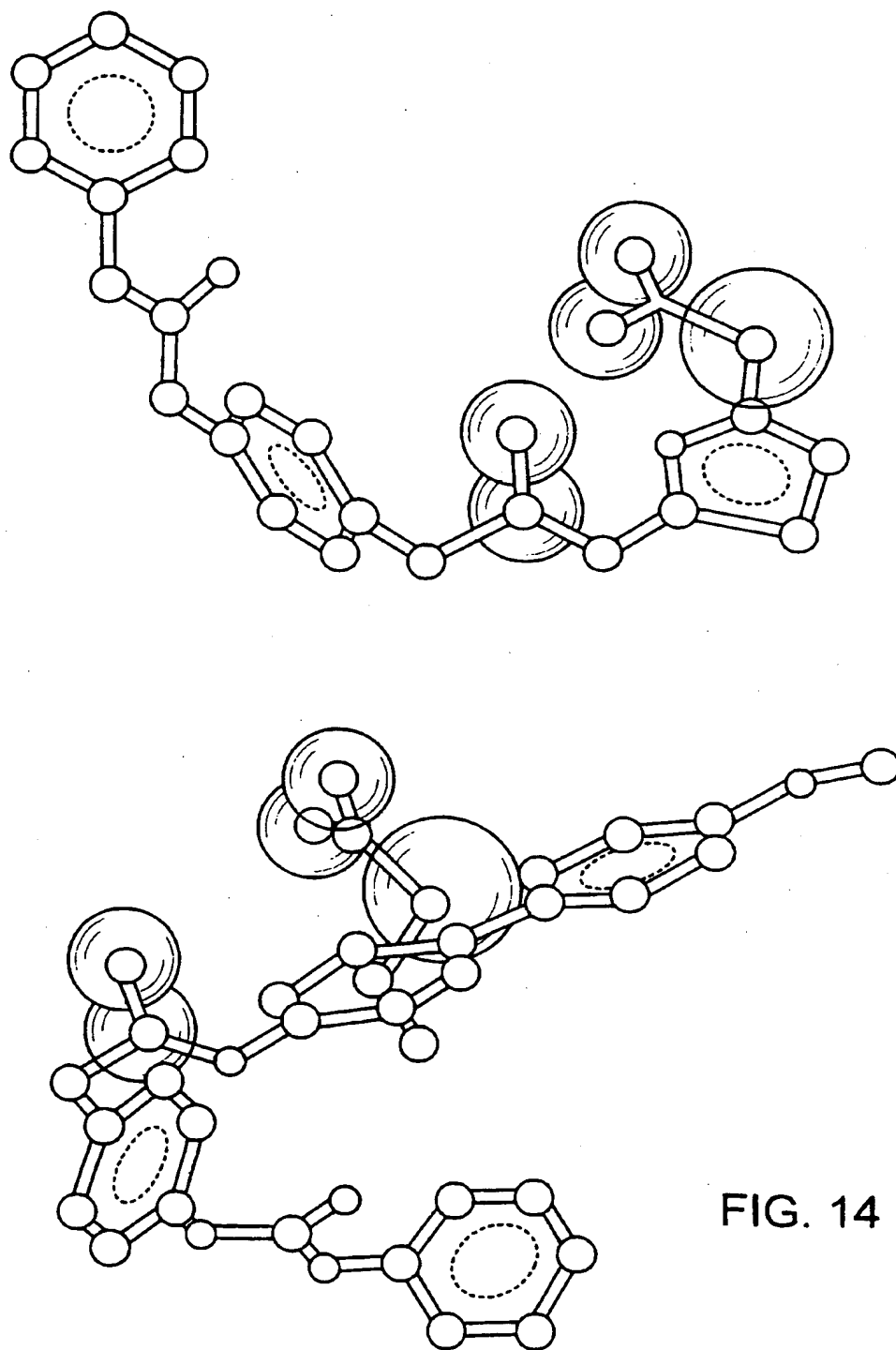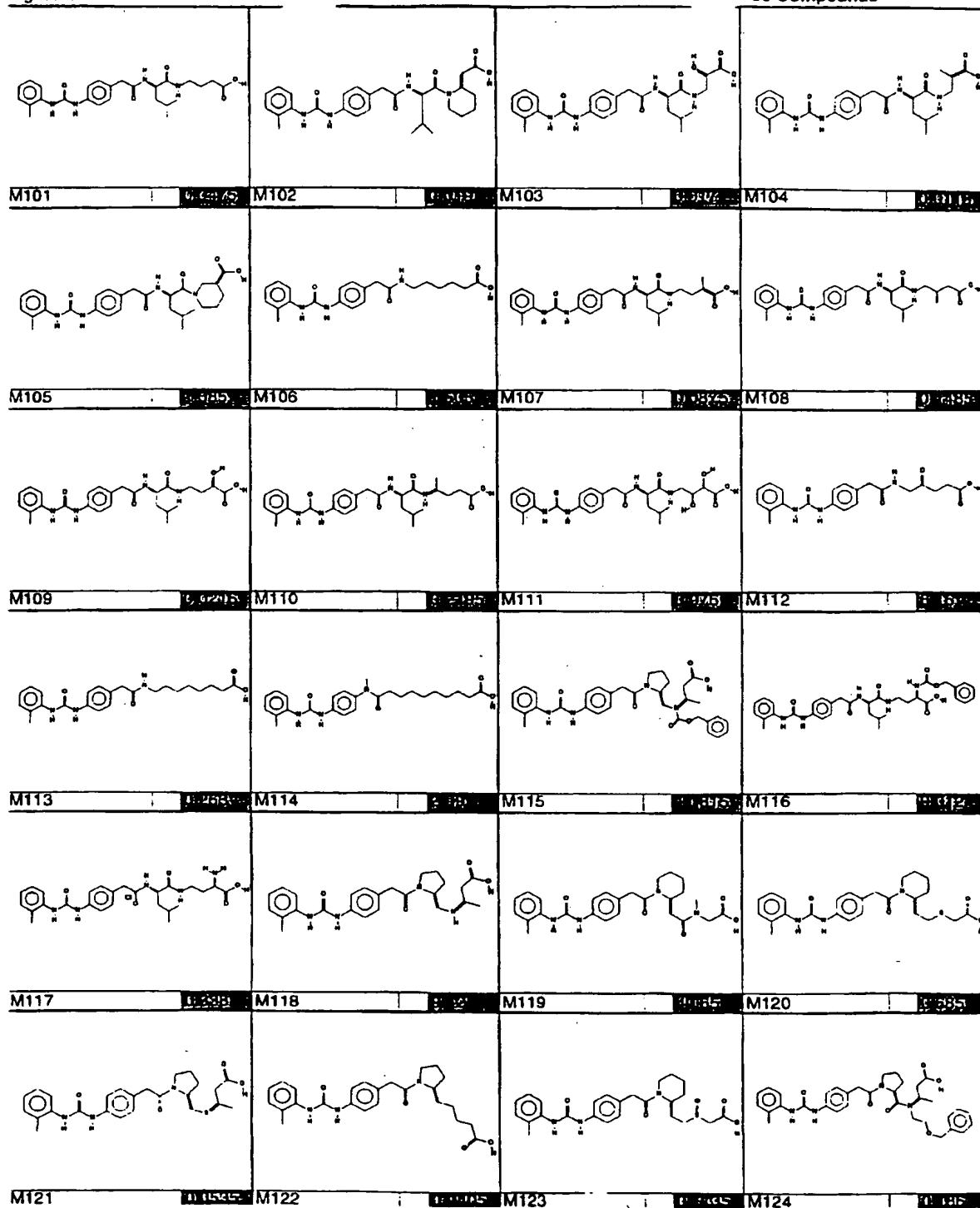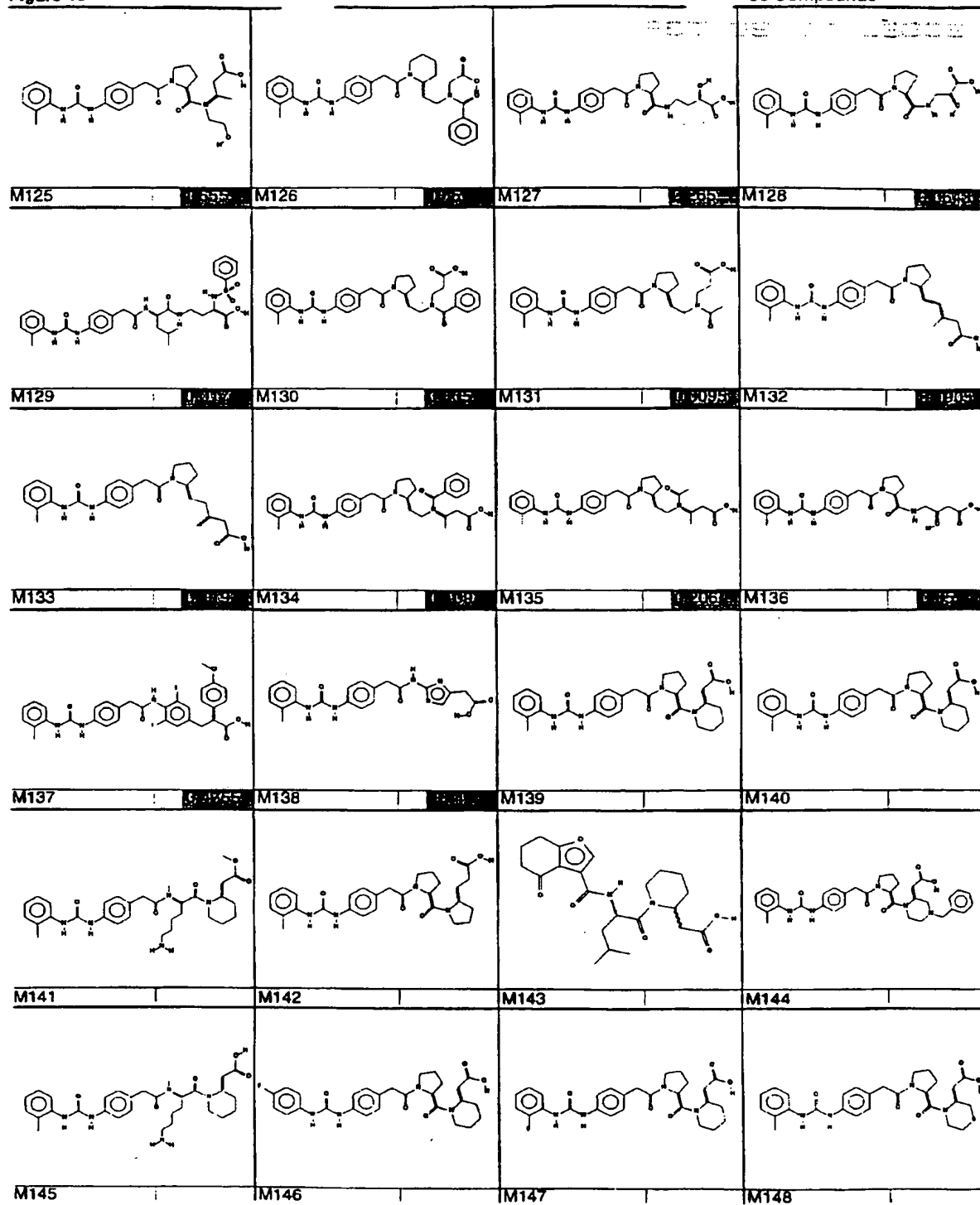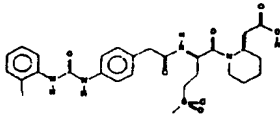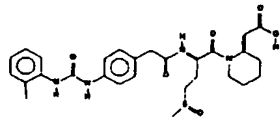
Figure 15

50 Compounds

| M149 | | M150 | | | | | |

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   G01N33/00   G06F17/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   G01N   G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 10140 A (UNIVERSITY TECHNOLOGIES INTERNATIONAL) 11 July 1991 see the whole document -----	1-70

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
31 October 1997	0 7. 11. 97

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1